

The wages of spin

Steps to increase the transparency of the procedures used to judge the safety of genetically modified foods are to be welcomed. The scientific advice on which such judgements are made must reflect the same openness.

The British government found itself treading on thin ice last week. In a bid to reduce public distrust of genetically modified (GM) food, it announced that consumer and ethics representatives will be appointed to two new “over-arching” bodies responsible for monitoring developments in human genetics and the agricultural use of biotechnology, respectively (see page 287). At the same time, it has resisted demands for broadening the membership of technical advisory committees to include non-scientist members.

The day before the new arrangements were announced, however, a letter was leaked to the media indicating the existence within the Cabinet Office of a ‘Biotechnology Presentation Group’ keen — among other things — to see editorial changes to the scientific report on which the government based its statement that there is “no evidence” that GM food is harmful, and to ‘clear’ its final draft. There is no evidence that the report was, in fact, modified at the group’s request. But the suspicion understandably remains that it might have been, particularly in the light of the presentation group’s stated desire to see prominence given elsewhere to the *benefits* of the technology.

Of course, the environmentalist groups who leaked the letter have little to complain about when it comes to ‘spinning’ news headlines. Much newspaper space, for example, was also devoted last week to attempting — on the basis of a letter to one such group — to portray Sir Robert May, the chief scientific adviser, as being at odds with the government on whether a moratorium should be placed on the commercial planting of GM crops. The government, although rejecting such demands, has refused to give the green light to such planting before the results of field trials have shown them to be safe; May merely said that he felt this would probably take several years to achieve.

Yet this does little to justify the government responding in kind, particularly at a time when public trust in official spokesmen is already low. Memories remain fresh of cabinet ministers issuing assurances that there was “no evidence” of a link between BSE and Creutzfeldt–Jakob disease. The refrain heard last week from supermarkets and their customers in response to similar statements about GM crops — “why should we believe them now?” — indicates the

size of the credibility gap that remains to be bridged.

It is both easy and tempting to make the press the scapegoat for this state of affairs. Few would deny that some recent headlines, largely fuelled by circulation wars among middle-market newspapers, have blatantly distorted the interpretation of scientific evidence about the potential hazards of GM crops. Indeed, the recent behaviour of such newspapers has prompted the House of Commons Select Committee on Science and Technology to propose that cases of scientific inaccuracy should be reported to the Press Complaints Commission.

This strategy might be appropriate if questions concerning the safety of GM crops could be reduced to straightforward scientific ‘truths’. But they seldom are. A more robust route would be to accept that the confusion between fact and interpretation is inevitable in a field as controversial as this, and to concentrate on providing people with the means to distinguish between the two.

One essential is to preserve the integrity of the peer-review process; papers that have been endorsed in this way — such as that published last week on the potential threats to the monarch butterfly from genetically engineered corn (see *Nature* 399, 214; 1999) — carry far greater legitimacy than those whose conclusions have not been carefully checked by scientific peers. A second is to ensure that the uncertainties in the scientific evidence, particularly in outlining potential long-term side effects, are given their proper weight when the scientific advice offered to ministers is turned into political practice.

From this point of view, the greater transparency promised by the government through the creation of its two broad monitoring bodies is to be welcomed. There remains a danger, however, that unless these are seen to be truly independent, not only of government (and industry) priorities, but also of a ‘scientistic’ mindset that limits the reflections of scientists and technical committees to that which is known, rather than that which can be envisaged, the process will remain within a straitjacket. After BSE, the consumer is no longer reassured by the statement, “there is no evidence that ...” The most appropriate forms of both government reassurance and scientific advice are now ones that take this fact and its implications actively into account. □

A sorry affair

A patent dispute involving allegations of scientific misconduct mars the image of the biotechnology industry.

Nature insists on the retraction of papers it has published that are subsequently shown to be false. But sometimes that is easier said than done, especially when co-authors of the original papers disagree over whether there is any fault at all.

Such occasions are, thankfully, rare. This issue contains an example that is even more unusual. At the heart of a billion-dollar lawsuit that has just entered the phase of jury deliberation (see page 289) are the contradictory statements of scientists as to whether a plasmid discovered at and patented by the University of California at San Francisco formed the crucial basis of a critical experiment in the early commercial success of the company Genentech, Inc.

The conflict can be seen starkly in separate letters addressed to this journal (see pages 297–298). Dennis Henner and current and former

colleagues from Genentech deny misrepresentation in a subsequent *Nature* paper describing these experiments, and using stolen materials. In sharp contrast, their co-researcher Peter Seeburg confesses to what he describes as “a technical inaccuracy”.

Nature is in no position to arbitrate. It is now up to a San Francisco jury to decide who has made the most convincing case, and even that judgement is unlikely to be final. It is some consolation that neither the paper itself — nor the value of the human growth hormone to which it has led — are in dispute. But the picture of commercial pressures lurking in the background does little credit to the public reputation of the biotechnology industry — and makes the adoption of guidelines for sharing research tools between federally sponsored researchers and industry (see page 291) even more urgent. □



Britain opens biotech regulation to greater public involvement

[LONDON] The British government is to make changes to the way in which developments in biotechnology are regulated. In particular, there is to be more open access to information on how decisions are made and more avenues for the public and special-interest groups to make their views known.

The changes, announced last week, are intended to address public concern about the effects of genetically modified (GM) foods on health and the environment. They are partly inspired by last week's publication of the results of a survey of the public's attitudes to the regulation of developments in the biosciences (see box on page 288).

It found that people feel there is too little control over research in the biological sciences. Respondents said they are not told enough about the progress of developments, and want government advisory bodies to be made up of people with a range of viewpoints.

The government announced that it is to set up two strategic bodies to oversee the work of the 17 committees involved in bioscience regulation. A Human Genetics Commission, which will advise on the use of biotechnology in health care, will replace the Human Genetics Advisory Commission.

The second body, the Agricultural and Environment Biotechnology Commission will assess the environmental effects of biotechnology in agriculture. The soon-to-be-established Food Standards Agency will be responsible for the safety of GM foods.

The government will also set up a national surveillance unit to monitor the effects of GM and other novel foods on health. Both of the new commissions will be made up of scientists, ethicists, representatives of consumer and environmental groups, and lay people, and will report to government ministers.

The changes have been welcomed by organizations representing scientists, such as the research councils. The Royal Society had told the government last year that bioscience regulation is too fragmented, and was among the first to recommend that an "over-arching" body be set up to oversee the work of technical and regulatory committees (see *Nature* 395, 5; 1998).

The arrangements have also been welcomed by the Nuffield Council on Bioethics, whose report on the ethical and social issues of genetically modified crops is published today (27 May). But Sandy Thomas, the council's director, says the public may question whether the new commissions are sufficiently independent from government.

The response from consumer groups, such as the Consumers Association, has been more cautious, and environmental organizations have dismissed the proposed commissions as inadequate. Both groups are concerned that the lay membership of technical regulatory committees will not increase.

Consumer groups want more public involvement in assessing individual GM products and processes. Julie Hill of the



Organic growth: environmental activists are leading the public's opposition to GM food.

Green Alliance, and a member of the government's Advisory Committee on Releases to the Environment, says that scientists on committees are too uncritical of the technology they are meant to be regulating.

But the government appears to have concurred with the views of industry, scientists and most of the House of Commons Select Committee on Science and Technology, that the approval of individual GM products should be left mainly to the experts.

At the same time the government says it accepts the need for increased public involvement in the regulatory process as a whole. The new commissions will be able to advise on the membership of regulatory committees and on how they work.

Consumer and environmentalist groups are also concerned that there is no indication whether the technical committees or the new strategic commissions will meet in public. Although existing committees publish agendas of meetings in advance and summaries of decisions taken, they meet in private.

The government has not agreed to open meetings, but the minutes of committee meetings and the arguments used as the basis for decisions should be published, it says. In addition, there are to be more public meetings, consensus conferences and workshops.

One official from the government's

Trade concerns dominate GM debate in US

[WASHINGTON] For many Americans, research published last week suggesting that pollen from genetically modified (GM) *Bt* corn might harm the larvae of monarch butterflies (see *Nature* 399, 214; 1999) was their first exposure to the environmental and health concerns from GM crops. But they are unlikely to call for greater control of GM technology.

The research was widely reported in the US media, appearing on national news broadcasts and in newspapers across the country. Margaret Mellon of the Union of Concerned Scientists (UCS), the most

vocal group opposing GM foods in the United States, predicts the attention will generate "a lot more opposition" to GM crops.

But industry groups and politicians are sceptical, believing that the public will not risk harming US agriculture and trade. Val Giddings, vice-president for food and agriculture at the Biotechnology Industry Organization, says European concerns about health and environmental safety are just "thinly veiled protectionism".

The UCS and the Environmental Defense Fund have called on the US Environmental Protection Agency to suspend approval

of *Bt* corn until the threat to monarchs can be assessed. But other environmental groups have remained quiet. In Washington, debate on GM foods is focused on trade issues.

On 13 May, 36 senators signed a letter written by John Ashcroft (Republican, Missouri) and Tom Harkin (Democrat, Iowa) calling on President Clinton to raise the issue of trade at next month's G-8 summit in Cologne, Germany, and at the November meeting of the World Trade Organization. Ashcroft's home state includes St Louis, home of the biotechnology company Monsanto. **Tony Reichhardt**

Office of Science and Technology says that, despite the demands from pressure groups, the public-consultation exercise did not find a widespread demand for more public involvement in the approval of individual GM products.

There is a fierce debate in Britain over whether GM crops should be grown commercially before the end of a four-year period of research into their environmental effects. Supporters of such a moratorium include the British Medical Association, the government's wildlife advisers English Nature, opposition political parties, environmental and consumer groups, and most of the media.

Research published last week, suggesting that monarch butterflies could be at risk from eating pollen from corn that has been genetically modified to increase resistance to pests (see *Nature* 399, 214; 1999), intensified calls for a moratorium, and exposed the tensions within government over the issue.

Many in government, including Michael Meacher, the environment minister, and Sir Robert May, the chief scientific adviser, while keen to point out the potential advantages of GM crops, are concerned that they could accelerate the erosion of biodiversity. They are reluctant to approve their commercial introduction until such concerns are allayed.

But others, notably Jack Cunningham, who chairs the ministerial committee on biotechnology, and Lord David Sainsbury, the science minister, are believed to be keen to promote the technology because of its role in the government's efforts at knowledge-based economic development.

Ehsan Masood

Britain backs biotech, seeks tougher regulation

[LONDON] Most of the British public is aware of developments in the biological sciences, but a significant proportion believes that research in some areas of biotechnology – notably cloning – is moving too fast, and that there is too little regulation.

These are among the findings to emerge from a public consultation on the biosciences commissioned by the government and published last week. They contributed to the government's decision to increase public involvement in overseeing developments in biotechnology (see page 287).

The survey – the largest of its kind to be undertaken in Britain – established the extent of public opposition to biotechnology, which has severely affected the government and industry's timetable for the introduction of genetically modified crops.

Researchers from the polling company Market and Opinion Research International (MORI)

interviewed 1,100 people face-to-face, and conducted six two-day 'focus group' workshops with 123 people. Respondents were asked five categories of questions, including the level of their awareness of the biosciences and their regulatory arrangements, and the issues they believe should be taken into account in any oversight process.

The public is aware of xenotransplantation and such issues, and is broadly supportive of the benefits of biotechnology, particularly in healthcare. But there is a feeling that information is being held back, particularly regarding answers to questions such as 'why is this development taking place?'. It is also less supportive of cloning and genetic modification in agriculture, and wants its views to be considered when decisions are made.

Many participants could not see the purpose of cloning, and remained concerned about the

possibility of human cloning, even when told it is illegal. They also felt they had been kept in the dark about it.

Similarly, respondents did not regard genetically modified food as being of benefit to society, and were unsure as to why it was being produced. The idea of using animal or human genes in plants for consumption was generally not considered acceptable. The most common reason given for GM food was 'to produce more food/have high yields/boost agriculture'. But this was followed closely by 'companies want to make money/have profit'.

When asked about bioscience regulation, 38 per cent believe that there is too little regulation of bioscience developments.

Hospital and family doctors came top of those most trusted to take regulatory decisions on the public's behalf (57 per cent). Retailers topped the list of those least trusted to take such decisions.

E.M.

South Africa reveals plans to make AIDS a notifiable disease

[CAPE TOWN] South Africa's minister of health, Nkosazana Zuma, has proposed that AIDS be made a notifiable disease in South Africa, in an attempt to reduce the spread of the infection which now affects one in ten South Africans.

The proposals would require doctors to inform local health authorities (anonymously) of persons diagnosed as having AIDS, as well as immediate family members and healthcare workers involved in the treatment of the patient. AIDS, rather than just 'natural causes', must be recorded as the cause of death on death certificates.

But the proposals have raised concern that, if the government were to go one step further and deny entry to South Africa to people who either have AIDS or are HIV-positive, this would pose a serious problem for delegates to the World AIDS Congress, scheduled to take place next year in Durban.

Announcing the proposals at a joint meeting last month with the health ministers from Namibia and Zimbabwe, Zuma said: "We can't afford to be dictated to

by human rights or AIDS activists. We want to know who is dying of AIDS, and relatives and partners must be notified. It is time we treated AIDS as a public health issue like TB. We don't go about treating that with secrecy."

No changes have been proposed for those who are HIV-positive, which does not need to be reported. The government has invited comments on the proposals before it promulgates the regulations.

Meanwhile, Ian Roberts, a special adviser on health affairs to Zuma, has issued a



Zuma: ending the secrecy over AIDS.

statement denying press reports that Zuma changed her mind about using the anti-AIDS drug AZT for the prevention of mother-to-child transmission (see *Nature* 396, 504; 1998). The reports appeared after the provincial government of Gauteng allowed the Chris Hani

Baragwanath Hospital in Johannesburg to accept a donation from UNAIDS to cover the cost of using the drug in research trials.

Mark Wainberg, chairman of the International AIDS Society, says he remains strongly opposed to any moves to boycott next year's conference in protest at the minister's stance. "There is consensus among its scientific community, including some of Zuma's most severe critics, that holding the meeting in South Africa will do far more good than harm," he says. "We should press ahead — unless something as horrible as a restrictive border-crossing policy were to be imposed."

In another development, Zuma has informed the US pharmaceutical company Bristol-Myers Squibb that the government could not endorse a proposal to invest \$100 million over the next five years on AIDS-related research in South Africa, including clinical trials on their products (see *Nature* 399, 96; 1999). The initiative was announced earlier this month without the prior consent of the government.

Michael Cherry

Charges fly in \$1bn hormone patent battle

[SAN FRANCISCO] Nine years of legal conflict came to a head this week as a jury began its deliberations at the end of a six-week trial to decide whether Genentech Inc. infringed a University of California patent for DNA for human growth hormone.

The trial, in federal court, has included testimony of a midnight raid 20 years ago to take DNA from a lab at the University of California at San Francisco (UCSF), allegations that at least one of the world's leading molecular biologists has been lying under oath, and charges that a key paper published in *Nature* in 1979 included false information, even though its conclusions remain valid.

In the lawsuit, filed in 1990, the university contends that Genentech used its patented DNA to perform experiments in spring 1979 that led to the creation of Genentech's blockbuster drug Protropin, a synthetic hormone used to treat growth disorders.

For this alleged infringement, the university is seeking \$400 million in back royalties and interest. It is also asking the judge to triple that amount to \$1.2 billion because of alleged wilful misconduct by Genentech.

If the jury finds in favour of the university, Genentech is likely to appeal, and the impact of any monetary judgement may be years away. But such a verdict would have a far greater psychological effect, as it would mean that what is arguably the most successful biotechnology firm of the genetic engineering revolution prospered because of misappropriated material.

Genentech executives deny any patent infringement, saying that its scientists constructed the DNA for human growth hormone independently in 1979, and that this was the DNA that was used in bacterial synthesis to create Protropin.

The trial has pitted former colleagues against each other. As Joseph F. Sambrook, director of research at the Peter MacCallum Cancer Institute in Melbourne, Australia, and an expert witness for the university, told



the jury: "Someone isn't telling the truth."

The most dramatic testimony involved Peter H. Seeburg, a former researcher at UCSF and Genentech who is now director of the Max Planck Institute for Medical Research in Germany, and David V. Goeddel, a former Genentech scientist who is now chief executive at Tularik Inc., a biotech company in South San Francisco. Seeburg described how, just before midnight on 31 December 1978, shortly after he left UCSF for Genentech, he slipped into the university, removed a sample of human growth hormone DNA from a lab, and took it to Genentech.

Genentech attorneys reluctantly acknowledged during the trial that Seeburg brought the UCSF material to the firm, but insist that it was not used to produce the hormone drug. After refusing repeated requests by the university to return the sample in 1979, Genentech paid the university \$2 million then to settle the dispute.

Seeburg told the court that this purloined material was used in spring 1979 to help Genentech develop the bacterial synthesis process used to produce Protropin (see pages 297–298). Seeburg and Goeddel were Genentech's lead investigators on the project at the time.

But Goeddel, along with other Genen-

tech colleagues, argues that the DNA from UCSF was not part of the landmark Genentech experiments in which *Escherichia coli* bacteria were genetically engineered to produce the synthetic growth hormone.

Seeburg maintained during the trial that he and Goeddel had a pact never to reveal the use of UCSF's DNA. But after years of silence and walking what Seeburg described as "a tightrope" during pre-trial depositions, he finally admitted to using it.

The trial audience was stunned when Seeburg testified that the *Nature* paper (*Nature* 281, 544–548; 1979) contained a number of "technical inaccuracies", including an outright falsehood, namely that a key plasmid used for the bacterial synthesis "did not exist".

Seeburg testified that the UCSF DNA was used instead, but was not referred to in the *Nature* paper because of the potential legal implications. He also said that an entire figure purporting to reflect the sequence of the Genentech human growth hormone DNA was a copy of the sequence of the UCSF DNA.

Genentech attorneys and Goeddel deny Seeburg's statements, with the lawyers challenging Seeburg's motives. The university licensed its patent for human growth hormone to Eli Lilly & Co. If the university is successful in its lawsuit against Genentech, Seeburg stands to earn nearly 10 per cent of recovered royalties, as he is a co-inventor of UCSF's human growth hormone DNA.

Genentech's attorneys also challenged Seeburg's character, saying his acknowledged problems with cocaine and alcohol during his early years at Genentech raise further questions about his veracity.

Scrutiny of 20-year-old Genentech notebooks of the critical experiments in 1979 was a crucial component of the case, with opposing sides interpreting the documents differently. None of Seeburg's notebooks was produced at the trial, but those of Goeddel and other Genentech researchers were.

Sambrook testified that his analysis of the notebooks and key experiments suggested that Goeddel and a research associate could not have completed the DNA experiments on the day in May 1979 entered in the notebooks, as there was not sufficient time in the day to complete the experiments in question.

Genentech attorneys dispute this, but one of Genentech's own experts, Judith L. Campbell, a professor of chemistry and biology at the California Institute of Technology, acknowledged under cross-examination that the notebooks did not prove that Genentech independently developed the DNA used in the experiments.

Campbell acknowledged there were "errors" in the *Nature* paper, parts of which she agreed were "misleading" or not supported by Genentech lab notebooks. **Rex Dalton**

Who's telling the truth about crucial plasmid?

In a letter to *Nature* (see pages 297–298), current and former researchers at Genentech say that they "categorically deny" accusations by their former colleague, Peter Seeburg, that data on the sequencing of a gene for human growth hormone published in a key paper in the journal in 1979 (see *Nature* 281, 544–548; 1979) were false, "and that he knew they were false when

the paper was submitted".

In response, Seeburg repeats an admission made during recent court proceedings (see above) that the work made use of a cDNA clone that had originated at the University of California, San Francisco, and writes "I deeply regret that, contrary to the principles of scientific endeavour, the *Nature* paper contains a technical inaccuracy".

The Genentech authors, who were the co-authors of the original paper and say that they stand by its content, say that Seeburg is being "intellectually dishonest" in claiming that it is permissible to make up data on the basis of "similar work", and in describing a non-existent plasmid as a "technical inaccuracy". Seeburg says "all scientific conclusions of the paper are correct".

Europe seeks greater role for women on key advisory panels

[MUNICH] Research ministers of the European Union (EU) last week gave their backing to plans by the European Commission to promote women in science by increasing their role on advisory panels and in fellowship programmes.

German research minister Edelgard Bulmahn, whose ministry has adopted broad measures to promote women in science, initiated the move to endorse the plans (see *Nature* 399, 186; 1999).

The plan sets a target of 40 per cent participation of women on the advisory, evaluation and monitoring committees of the Commission's fifth Framework programme of research (FP5), launched earlier this year. They also aim for a similar level of female participation in the FP5's Marie Curie programme of fellowships for young researchers to work in another EU country, and include strategies for coordinating national efforts to promote the role of women in science.

The commission expects the fellowship target to be relatively easy to achieve, as women made up more than a third of the Marie Curie fellows funded in the fourth Framework programme (FP4), where no special measures for gender equality were taken. It is encouraging more applications from women through an information campaign to universities.

Meeting the target for committee membership could be more difficult. Less than ten per cent of the 2,000 or so evaluators for the FP4 were women, and the committees that will evaluate the first round of FP5 proposals must be drawn from a database of self-nominations from the research community. Yet less than 15 per cent of the entries on the database are women.

The commission is encouraging more women to nominate themselves for the database. It faced this problem last year in setting up the FP5's strategic external advisory groups. For these, women made up only nine per cent of names put forward by EU member states and 13 per cent of self-nominations. Despite this, it selected 27 per cent female membership, and 40 per cent of the groups are chaired by women.

Meeting in Berlin last week, the research ministers passed a resolution saying they recognized the under-representation of women in research and believed it should be tackled at regional, national and European levels. They also promised to ensure that statistics are gathered and made available, to "facilitate the development of appropriate policies at national and Community levels".

Alison Abbott

Australian geologists left reeling by budget cuts ...

[SYDNEY] The Australian Geological Survey Organisation (AGSO) is to lose one-fifth of its funding and staff numbers as a result of budget cuts announced after the 1999–2000 budget earlier this month.

Among those hit is the Department of Industry, Science and Resources, which shed A\$151 million to a total budget of A\$2,333 million (US\$1,547 million). An increase of seven per cent to support industrial innovation by industry has coincided with greater cuts in other programmes, including AGSO.

The organization experienced similar budget cuts three years ago, losing full-time access to a survey vessel but gaining a new building (see *Nature* 381, 547; 1996). Staff numbers had reduced to 490 this year and are now expected to fall to around 400.

According to an internal memorandum, AGSO is balancing its books by phasing out land surveys and some other scientific programmes, sacking all its palaeontologists and reducing staff in its minerals division by a quarter.

Senior research scientists will have to compete with administrators for a reduced number of executive posts. And closure of the stores in its new building means that AGSO will lack in-house equipment to support fieldwork: projects will have to buy or lease gear and transport from their own reduced funding.

One geologist says that AGSO had prided itself on becoming one of the first branches of the public service to introduce promotion based on merit rather than seniority. Now, he says, "nine of those promoted to principal research scientist out of 14 in the minerals division are being given no choice but to accept redundancy. Staff are devastated."

A government official admits that the cuts are "unfortunate", but says they are a direct result of the ending of two major projects, the National Geoscience Mapping Accord and the Law of the Sea Project. In future, AGSO's operations will "focus more on offshore petroleum work and Australia's marine jurisdiction work".

But Malcolm Walter of Macquarie University in Sydney describes the closure of land surveys as "a devastating blow". Bob Day, president of the Australian Geoscience Council and a former chief geologist in the state of Queensland, says: "The losses will only exacerbate the crisis for geoscience with up to half of Australia's 8,000 geoscientists now out of a job."

Peter Cullen, president of the Federation of Australian Scientific and Technological Societies, is calling for a strategic review of government investment in geoscience. "The government is letting the nation's skill base slip through our fingers at the first signs of a downturn," he says.

Peter Pockley

... while New Zealand research gets thin rations

[SYDNEY] With a general election looming, New Zealand's minority National Party government has increased science and technology spending by NZ\$14.8 million to NZ\$426.2 million (US\$226 million) in its budget for 1999–2000, keeping funding slightly ahead of inflation.

But this increase will make no impact on NZ's ranking near the bottom of the Organization for Economic Cooperation and Development's league tables. A target set in 1996 for the research and development budget to grow annually, from 0.53 to 0.8 per cent of gross domestic product by 2010, appears to have been shelved.

A New Economy

Research Fund, worth NZ\$5.6 million, has been introduced to help generate new technologies, and some grant schemes have received small increases. But the university sector is upset that there was no restoration of a cut of NZ\$95 million made during last year's economic crisis.

Graeme Fogelberg, chair of the NZ Vice-Chancellors' Committee, said the cuts would: "rip the heart out of postgraduate education to fund undergraduate courses and a 'contestable research' fund," worth NZ\$20 million next year.

The budget was preceded by an announcement of the cabinet's blueprint for research and development.

These funding guidelines are based on 14 'target outcomes' developed as part of the Ministry for Research, Science and Technology's controversial Foresight project (see *Nature* 398, 250; 1999).

George Petersen, president of the Academy Council of the Royal Society of New Zealand, is relieved that the importance of basic research is specifically mentioned in the outcomes. But he is disappointed in the lack of answers to pressing questions about funding levels and distribution.

A move to run all government-funded research using the Foresight model is said to have been shunned during budget planning by other ministries.

P.P.

NIH strives to keep resource sharing alive

[WASHINGTON] The US National Institutes of Health (NIH) last week called on universities to "take every reasonable step" to ensure the free sharing of research tools between scientists.

Issuing guidelines to university officials who negotiate licences and agreements for sharing research tools, the NIH said such officials should do everything possible to make research tools discovered by NIH-funded investigators freely and broadly available. It says this mandate does not clash with a 19-year-old law that opened the door for universities to issue exclusive licences on government-funded inventions.

The guidelines urge universities to speed up the transfer of tools to academic scientists elsewhere, and to private companies that are not using them for direct commercial purposes. They also say that exclusive licences for research tools should "generally be avoided", except when the licensee plans to make the tool widely available at an affordable cost, or when the licensor keeps the rights to do so.

Institutions are urged to avoid patenting research tools. The guidelines say these rarely require patent protection, as further research, development and private investment are not needed to realize their usefulness. The "indiscriminate" patenting of such tools is "antithetical" to the goal of getting taxpayer-funded inventions out to the public.

The guidelines were developed by the NIH's Office of Technology Transfer on the recommendation of a working group that reported last year to NIH director Harold Varmus (see *Nature* 393, 505; 1998).

The NIH has repeatedly expressed concern about impediments to the sharing of research tools, and the guidelines mark the agency's first formal written policy on the issue. They are due to be published in the Federal Register this week, and will be open for public comments for 90 days before a final version is drawn up.

The guidelines chart a fine line between encouraging the sharing of research tools and the provisions of the Bayh-Dole Act, a 1980 law that gave universities ownership of government-funded inventions and encouraged them to license them in order to commercialize them for public benefit.

One NIH official who helped draft the guidelines says the Bayh-Dole Act should not be read as an eleventh commandment — "thou shalt patent and exclusively license" — that precludes the liberal sharing of research tools. "Commercialization is a good thing, but we want [universities] to commercialize without encumbering future research," the official says. "It's NIH's view that you can accomplish both with strategic licensing."

The guidelines call for "thoughtful,

strategic" implementation of the Bayh-Dole Act on a case-by-case basis, in order to strike a balance between fostering product development and making sure researchers have access to the cell lines and clones they need.

Cases in which a technology needs to be licensed exclusively in order to develop it as a product, but where it is clear that it should be made widely available as a research tool, will have to be worked out with "creative licensing", adds the NIH official. "This is a road map but not a solution for every problem."

Rebecca Eisenberg, a patent-law expert at the University of Michigan Law School in Ann Arbor and chair of last year's working group on research tools, points out that the guidelines do not exclude the possibility that "real winner[s]" can be licensed on lucrative terms. But she thinks the NIH is "trying to stop that from being the default position".

The guidelines also urge technology-transfer offices to scrutinize the agreements under which they obtain research tools from other non-profit institutions or industry. Officials are "expected to avoid" signing agreements likely to keep them from being able to broadly disseminate further tools that might arise from the research. Excessive publication delays, requirements for editorial control or pre-publication approval, and withholding of data, are all "unacceptable", the guidelines say.

The guidelines were praised by university technology-transfer officials, many of whom were consulted during their drafting, but some aspects are likely to raise concern at institutions where the Bayh-Dole Act has been read as a licence to make as much money as possible from technology transfer. Many institutions prize money generated in this way, as its uses are unrestricted.

Other university officials say that it is not loss of revenue, but loss of control, that

worries them. Fred Erbis, director of the Office of Intellectual Property at Michigan State University in East Lansing, says that the NIH's call to institutions to avoid patenting research tools "bothers me".

The university is still smarting, he says, from an incident seven years ago, when genetic material was loaned under very loose terms to a company that was sponsoring unrelated research at the university. That company patented it, with the result that the molecular biologist at the university who is the inventor can't share it with colleagues in the laboratory next door.

Institutions receiving NIH funding for their investigators are not required to adopt the guidelines. But the agency exhorts both academia and industry to implement them.

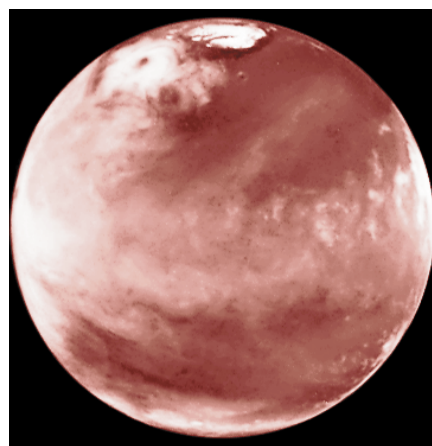
That can't be assumed in the private sector. "Our position and the NIH's are not necessarily the same," says David Schmickel, the patent and legal counsel for the Biotechnology Industry Organization. "We'll be considering the guidelines in the light of our best interests, which also have a significant public-health benefit."

Some scientists feel that the NIH should go further than issuing guidelines. Michael Green, a Howard Hughes Medical Institute investigator at the University of Massachusetts Medical Center in Worcester, says that, while they go "in the right direction", they "will not be sufficient to solve the problem".

"They should mandate them for all NIH-funded investigators and then [the NIH should] take some kind of responsibility for enforcing them," Green says. After several years of failed attempts to obtain three published plasmids and one published cell line from fellow investigators, he says he has now given up.

The guidelines are available on the web at www.nih.gov/od/ott and comments may be sent to nihot@od.nih.gov **Meredith Wadman**

Hubble spots giant cyclonic storm on Mars



[LONDON] A giant cyclonic storm was seen raging in the northern polar regions of Mars last month by the Hubble Space Telescope. The storm, which was 900 miles long and 1,100 miles wide, is the largest ever detected on the planet. It was largely dust-free, and is believed to have been composed of clouds of water ice, similar to storm systems on Earth. The eye of the storm was 200 miles across.

The storm can be seen to the left of the polar ice cap in the picture. The dark spot above the cloud is the extinct volcano Ascreus Mons, which is 16 miles high and 250 miles across. The storm, detected by Hubble's Wide Field Planetary Camera, has not been seen since and may be over. **E.M.**

JIM BELL/STEVE LEE/MIKE WOLFF/NASA

Ethicists urge funding for extraction of embryo cells

[WASHINGTON] A US presidential commission is poised to recommend that the government should pay not only for research on stem cells extracted from human embryos left over at infertility clinics, but also for the process of extraction.

The recommendation appears in the draft of a report that the National Bioethics Advisory Commission (NBAC) hopes to finalize at the end of June. It conflicts with current legislation that bars the government from funding research in which human embryos are destroyed or discarded.

The report is likely to inject more controversy into a heated debate. Abortion opponents and their allies on Capitol Hill are contesting a less radical position already taken by the Department of Health and Human Services. The department interprets the current law as allowing the federal funding of stem-cell research, although not the extraction of the cells from embryos.

But the bioethics commission argues that both the extraction of the stem cells — which involves destroying the embryo — and research on them are “ethically acceptable for federal funding”. It calls on Congress to repeal parts of the law.

“The ban conflicts with several ethical goals of medicine, especially healing, prevention and research,” says the draft report. “Conservatives who accept that killing a fetus is permissible where it is necessary to save the life of the mother should agree with liberals that it is also permissible to destroy embryos where it is necessary to save people.”

Thomas Murray, president of the Hastings Center, a bioethics think-tank in Garrison, New York, and a member of the commission, says its conclusions were influenced by



Murray: ‘enormous medical potential’.

the medical promise of embryonic stem cells, whose isolation was announced last November (see *Nature* 396, 104; 1998 & 397, 185; 1999).

The cells are thought to have the capacity to differentiate into virtually all types of cells, possibly leading to therapies for a wide range of diseases, from Parkinson's to juvenile diabetes. According to Murray, commission members weighed this potential value against the fact that thousands of frozen embryos stored at infertility clinics are bound for destruction anyway. He says: “We are looking at research that has the potential to benefit many millions of Americans and many more people around the world.”

The draft says that government-financed derivation of stem cells from aborted fetuses, already permissible under federal law, should continue. But it draws a line at the creation of embryos for the purpose of generating stem cells, citing no “compelling” reasons for the government to finance this.

It also advises that couples should not be approached to donate excess embryos — more are typically created during fertility treatment than are implanted — until they have ceased fertility treatment. And it says that national and local oversight of research protocols will be “crucial”.

Eric Meslin, NBAC executive director, emphasizes that the report is not final, and says that “further and intense discussions” among commissioners are still possible. But Murray says the conclusions are unlikely to be substantially changed.

The National Institutes of Health (NIH) is already finalizing guidelines in which it says its investigators will be able to conduct research on embryonic stem cells (see *Nature* 398, 551; 1999). It is not clear what will happen when the NIH begins such funding in the face of a congressional ban whose authors, led by Congressman Jay Dickey (Republican, Arkansas), insist that it forbids stem-cell research.

Richard Doerflinger, a spokesman for the National Conference of Catholic Bishops, says the NBAC's recommendations come as no surprise because the commissioners were appointed by President Bill Clinton, who has supported research with excess embryos. But Doerflinger says: “There are more people in Congress than on the commission that recognize the human embryo as having a right to life. So we expect a different conclusion there.”

Doerflinger agrees with one NBAC conclusion, however: “They find it very unconvincing to divorce the use of the cells from their harvesting. The commission is willing to bite the bullet and have an honest debate on what the law should be.”

A poll released last week indicates that three out of four Americans are willing for the government to fund stem-cell research. In a survey of 1,005 adults, 74 per cent said they favoured federal funding after having been read a statement that “researchers believe that stem cells can be developed into replacement cells to cure diseases such as diabetes, Parkinson's, Alzheimer's, cancer, heart disease” and others.

The poll was commissioned by the Patients' Coalition for Urgent Research, a group of 27 medical research and disease advocacy organizations launched last week to lobby for funding.

Meredith Wadman

Europe's molecular biologists could join global e-journal plan

[PARIS] The European Molecular Biology Organization (EMBO) may join the initiative of the US National Institutes of Health (NIH) to launch a global website to centralize much of the biomedical literature and make it freely accessible. EMBO executive director Frank Gannon met NIH director Harold Varmus recently for talks.

The meeting may be the first step in the internationalization of the ‘E-Biomed’ project (see *Nature* 398, 735; 1999). The EMBO council is now considering the possibility of joining the NIH in an interim governing board and offering its peer-review services.

Some observers predict that, if such a board can attract the major stakeholders, it would be sufficient to get the initiative under way. That would avoid the need for an international consultation that might see the project derailed by publishers and professional societies with interests in maintaining the current print journals system.

Separate plans to establish a preprint server in biomedicine are already being discussed by the *British Medical Journal* and HighWire Press, a not-for-profit outfit set up in 1995 by Stanford University Libraries and Academic Information Resources to help universities and societies publish on the web at low cost.

“I think we are going to go forward anyway [despite ‘E-Biomed’] on the grounds that a thousand flowers may bloom,” says Michael Keller, head of HighWire Press. “At some point there will be a collapse into one or several servers,” he predicts.

Richard Horton, editor of *The Lancet* medical journal, says that he is in principle ready to support Varmus's proposal. “I find ‘E-Biomed’ a very welcome stimulus for debate. I'm delighted to see it,” says Horton. His vision is of the bulk of the primary literature being freely accessible, with only the top journals surviving as commercial enterprises.

But Horton is concerned that the proposed global database may accentuate the Anglo-Saxon domination of publishing, and result in discrimination against scientists from non-English-speaking, and developing, countries.

Vitek Tracz, chief executive officer of the Current Science group, says his company will also launch a preprint server (with optional peer review) this summer. The company has decided to offer all primary papers in medicine and biology free online, and to reap profits from review journals. Tracz is a keen supporter of ‘E-Biomed’, and is discussing how his company could participate.

Declan Butler

NASA beefs up life science involvement

[WASHINGTON] The US space agency NASA has appointed Nobel prizewinning biochemist Baruch 'Barry' Blumberg to head its new Astrobiology Institute, and neuroscientist Kathie Olsen as the agency's new chief scientist. Both moves will strengthen NASA's position in the biological sciences.

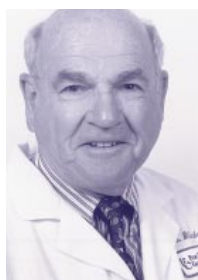
The 73-year-old Blumberg, who won the 1976 Nobel prize in medicine for his work in developing the hepatitis-B vaccine, teaches medicine and anthropology at the University of Pennsylvania and is an adviser to the Fox Chase Cancer Center in Philadelphia.

He will move to the Ames Research Center near San Francisco in September to head NASA's year-old 'virtual institute', a collective of scientists from 11 institutions investigating the origin of life and its possible existence beyond Earth.

Although he became aware of the field only a few years ago, Blumberg is among a handful of Nobel prizewinners who have been enthusiastic participants in a series of workshops at Ames to lay the foundations of astrobiology.

Blumberg says his unfamiliarity with previous debates on the topic may be an advantage: "I can be non-partisan." His appointment raises hopes that the institute will be the kind of intellectual incubator envisaged by NASA administrator Daniel Goldin.

The search for a director "took longer



Blumberg: will lead a 'virtual institute'.

Olsen, holder of a series of administrative jobs at the National Science Foundation since the mid-1980s, lacks Blumberg's status as a researcher. But her appointment fulfils Goldin's long-standing promise to appoint a biologist as his chief science adviser.

Traditionally, the position has had little authority, and has been vacant for almost three years. Budget and programme responsibility lies with NASA's three science offices: space science, Earth science, and life and microgravity science. Part of Olsen's job will be to broker differences among the strong personalities who head these offices.

But she will have Goldin's support as she attempts to raise the profile of life-science research at NASA. The agency hopes to increase spending in two areas: astrobiology and 'bio-astronautics', a blend of operational

than we had hoped", says the head of one member institution. High-speed video-conferencing capability has also been slow to materialize, hindering the Astrobiology Institute's role as an experiment in long-distance collaboration. Now the pieces appear to be falling into place.

medicine for astronaut crews and research into human adaptation to space flight.

Frank Sulzman of the office of life and microgravity sciences recently told NASA's external advisory committee that the agency wants to shift from simply "taking inventory" of activities in biology to augmenting the budget for ground-based laboratory work and experiments in space.

For example, NASA will soon announce the winners of grants in "biology-inspired" space technologies. These could range from advanced life-support techniques to 'smart' materials based on living systems.

Blumberg credits Goldin with the vision to link NASA's space research with advances in genomics and other life sciences. After decades of wondering about the existence of life in the Universe, he says, scientists can now conduct experiments and collect real data. A believer in the value of inductive reasoning, he says astrobiology will have the advantage of being "unencumbered by hypothesis".

Running the institute, with its "fairly complicated organizational scheme", will be a challenge, he admits. But he looks forward to working with astronomers, biologists, chemists and other researchers, and to attracting collaborators not just from US institutions, but from other parts of the world. "My big job," he says, "is to get scientists interested in the field." **Tony Reichhardt**

Tokyo meeting airs problems of international space collaboration

[TOKYO] Political and legal constraints remain serious obstacles to collaborative space projects, according to a meeting in Tokyo last week of space scientists from the United States, Europe and Japan. These include different approaches to liability waivers and sensitivities to the international transfer of space hardware.

The three-day meeting — attended by representatives from the European Space Science Committee (ESSC), a body associated with the European Science Foundation, the US National Academy of Science's Space Studies Board and the Space Research Committee (SRC) of Japan's Science Council — aimed to identify the key factors in facilitating future international missions.

Participants pointed out that, although international collaboration is increasingly important as space-science budgets decline, this may be discouraged by a shift by the US space agency NASA and the European Space Agency towards smaller, cheaper missions.

A report on US-European space collaboration, released last year by the ESSC, recommended the use of eight criteria to assess whether future international missions

are likely to be successful. These included scientific support through peer review and appropriate procedures for data handling (see *Nature* 394, 112; 1998).

"We are now trying to get Japan into the picture by focusing on the lessons of our past and ongoing collaborations," says Len Culhane, director of the Mullard Space Science Laboratory at University College London and chairman of the ESSC.

"There are issues arising from differences in our administrative systems. But the gaps [between the partners] will have to be filled to make international collaborations more successful."

According to scientists involved in international missions, such as the Yohkoh solar mission and the X-ray astronomy mission ASCA, collaborations between Europe, Japan and the United States have been scientifically very successful, even though language and cultural differences — as well as Japanese scientists' reluctance to release data — initially caused concern.

However, Japanese researchers expressed concern at last week's meeting over legal differences between Japan and the United States on liability and export matters. They

cited tensions between NASA and Japan's Institute of Space and Astronautical Science (ISAS) during the development of GEOTAIL, a satellite for investigating the structure and dynamics of the tail region of the magnetosphere.

Although NASA lawyers had required that each party bear its own risk of participation in a joint space activity, Japanese law does not permit this.

"The issue remains unresolved, and casts a long shadow on future collaboration, but this has to be handled at a higher, political level," says Atsuhiko Nishida, director of ISAS and chairman of the SRC.

Roger Anderson, an astrophysicist from the University of Iowa and a member of the US Space Studies Board, says there were problems with the international transfer of space hardware on both sides of the Pacific during the development of GEOTAIL. This caused problems with endorsing instruments and "discomfort" for scientists.

"What is most concerning is the impact of current US export regulations not only on hardware but also on the distribution of, and access to, scientific databases," he says. **Asako Saegusa**

US academies warn against curbs on visiting scientists

[WASHINGTON] The leaders of the three main US scientific bodies issued a joint statement last week warning that “inappropriate restrictions” on foreign visitors to the Department of Energy’s national laboratories “could weaken the nation’s role as a key player in the international scientific community” and risks retaliation by other countries.

Bruce Alberts, president of the National Academy of Sciences, William Wulf, president of the National Academy of Engineering, and Kenneth Shine, president of the Institute of Medicine, mindful of the recent controversy over allegations of spying at Los Alamos National Laboratory, say that they recognize the importance of protecting US national security interests, and announced a “fast track study” on how to maintain scientific leadership while doing so (see *Nature* **399**, 189; 1999).

But they warn that “new restrictions on interactions with foreign scientists ... would almost certainly lead to reciprocal restrictions on US scientists’ access to foreign laboratories, thereby greatly reducing our knowledge of and potential influence on other nations’ activities”.

Separately the council of the American Physical Society has pointed out that foreign visitors and students have made enormous contributions to American science. “Any negative characterization of scientists on the basis of ethnic or national origins is destructive to science,” says the society.

Frühwald lands top job at Humboldt Foundation

[MUNICH] **Wolfgang Frühwald**, former president of the Deutsche Forschungsgemeinschaft (DFG) and professor of the history of modern German literature at the University of Munich, has been appointed president of the Alexander von Humboldt Foundation. He will succeed Reimar Lüst, an astrophysicist, in November.

The Humboldt Foundation provides long-term research grants for foreign scientists to work in Germany. It is the first time that the president of the foundation will be from outside the natural sciences.

California’s undergrads get stuck into research

[SAN DIEGO] The eight campuses of the University of California are offering growing opportunities for undergraduate students to participate in a variety of research projects,

according to a report presented last week to the university’s board of regents.

A survey of students in 1997–98 found that at least 2,000 undergraduates took part in organized research projects in fields such as epidemiology, robotics, hydrology, photodynamic cancer treatment, gamma-ray astronomy and particle physics. The total number of undergraduates involved in research is believed to be much higher, said officials.

US call to expand ocean monitoring system

[WASHINGTON] A council of 12 US federal agencies involved in ocean research has recommended to Congress an expanded, integrated ocean monitoring system similar to the network used for weather forecasting. The report, from the National Ocean Research Leadership Council, was backed by a letter from 1,800 marine researchers, who wrote that “our national need for ocean observation is grossly underserved”.

The plan calls for an augmentation of the current network of platforms on satellites, buoys and ships for monitoring open ocean and coastal areas. It recommends a series of pilot projects, and development of standard data products. Details are available on: <http://core.cast.msstate.edu/NOPPobsplan.html>

Japanese ministries in new SNP mapping bid

[TOKYO] Japan's science-related ministries are planning to set up a project to identify and analyse single-nucleotide polymorphisms (SNPs), the variations in the human genome that could be linked to common diseases. The project is seen as an attempt by the government to join the international move to map SNPs (see *Nature* 398, 545–546; 1999).

Following an interministerial meeting held on 14 May, five ministries and agencies, including the Science and Technology Agency, the Ministry of International Trade and Industry and the Ministry of Health and Welfare, made plans to incorporate funding for the SNP project in this year's supplementary budget, the details of which will be released in the autumn.

India and Russia in joint effort on supercomputers

[NEW DELHI] An Indian-made supercomputer, Param-10000, will be at the heart of an Indo-Russian Computer Centre to be set up in Moscow, according to Indian science ministry officials. The centre is part of a long-term programme of scientific collaboration between Russia and India. A

large computing facility will be built around the computer, says Y. P. Kumar, the programme's coordinator.

The centre — a 50–50 venture between the two countries, but to be controlled by India — is likely to be commissioned in September this year. Param-10000 was designed and built by the Centre for Development in Advanced Computing in Pune. A smaller version of the computer was sold to Russia five years ago, but the Moscow centre will house the latest model.

Australia's top scientist wants more 'awareness'

[SYDNEY] Robin Batterham, a chemical engineer and managing director for Australian research and technology development for the mining company Rio Tinto, was appointed last week as part-time chief scientist in Australia. Batterham is a former chief of minerals and process engineering for the Commonwealth Scientific and Industrial Research Organization.

Batterham says his top priority is “to get the level of science awareness in the community right” by working at all levels with schools, professional and industrial bodies, government and the public. He will run the prime minister's Science,

Engineering and Innovation Council, and has spoken frequently on integrating the roles of science and technology in the innovation process — a problem in Australia (see page 290).

Islamists seek scientists for war against Russia

[MOSCOW] The Russian government may be attaching a low priority to science, but supporters of an Islamic state in Chechnia and Dagestan are showing special interest in the potential contribution of scientists to their cause. Magomed Tagaev, leader of the anti-Russian movement in the Caucasus, has published a book calling for preparations for a “long war” against the Russians and emphasizing the importance of building up military bases and creating centres for scientific research.

“We need to organize small but very effective groups able to locate specialists of the highest qualification, to hire them and, if this is impossible, to kidnap them and make them work for us,” writes Tagaev.

Among the specialists most needed by the proposed new Muslim state, the Caucasian Confederation, he names physicists, chemists and mechanics. Tagaev also promises to launch a “great march on Moscow” within the next few months.



The full text of items on this page — and further details and background information about the Unesco/ICSU World Conference on Science, to be held in Budapest from 26 June to 1 July — can be found on *Nature's* website at <http://www.nature.com>

How expertise could add up to help for developing countries

[NICE] Developing countries need a capability in cutting edge scientific research — including mathematics — to handle difficult social, economic and political choices, according to Claude Lobry, director of the International Centre for Pure and Applied Mathematics (CIMPA) in Nice, France.

"The problems of the poor countries — health, malnutrition, pollutants, infrastructure, energy and so on — demand rapid decision-taking," says Lobry. "Their development requires a massive mobilization of scientific knowledge to enable them to say: 'the following steps may be considered, their implementation will take so long, these will be the costs incurred, and so on'."

Researchers must play a key role in setting up committees of experts who are competent and trustworthy, argues Lobry. Only those actively involved in the informal network of international research can suggest individuals who are competent to deal with particular issues. "The most important thing is that they be outstanding in their own field, and therefore have access to the best sources of information."

Lobry says that any significant social issue in a developing country has economic and political implications which affect industrialized countries, and that these countries cannot therefore be left to set up expert committees alone.

"Imagine a country with vast stretches of desert, willing to hire this land out to developed countries as a dump site for their toxic waste," he says. "The safety conditions and a fair remuneration for the service must be discussed. Can the country providing the service be expected to put its blind trust in the experts of the country which is buying the service? Obviously not."

The situation would be completely different if this country had a team of efficient physicists, who could draw up an opinion based on the quality of the expertise provided. The South must therefore urgently acquire a research body capable of tapping into the global corpus of scientific knowledge, and it is the duty of the North to assist them in this.

This is even true of mathematics, says Lobry. "Mathematical skills are lost or blunted unless they are maintained by research. Mathematicians must work at their own subject if they are to intervene effectively in other disciplines."

Full text: <http://helix.nature.com/wcs/c17.html>

Swedes call for emphasis on access to knowledge

[LONDON] Unesco is being prompted by Sweden's national commission for the UN agency to play a more proactive role in the World Conference on Science by putting its weight behind calls for full public access to scientific knowledge. The Swedish commission would also like to see social scientists play a greater role at the Budapest conference than is currently planned.

Public access to knowledge, women in science, and investment in Third World universities have emerged as the three priority areas for Sweden's delegation to next month's conference. Swedish delegates are expected to press Unesco to end its position of neutrality and "take a stand" by supporting the interests of the developing world.

Most of Unesco's member countries are in the developing world, says Anders Falk, general secretary of the national commission. "Unesco should support them," he adds. For example, he says, "they need access to knowledge. Without this, they will never be able to develop." Unesco, says Falk, should oppose what he calls the "increasing privatization of knowledge".

Sweden has been at the forefront of developed countries in demanding that investment in basic research and higher education should be considered integral to the process of development. The country has one of the largest overseas research budgets, concentrating its aid on research activities in east and southern Africa.

Sweden's strategy for the Budapest conference has been agreed after three preparatory meetings, the last of which took place last week at Uppsala. The conference has generated considerable controversy, says Falk. Many delegates attending the preparatory meetings have voiced criticisms of the conference programme and of draft versions of the two final documents — a declaration and an 'agenda for action' — that the conference will be asked to adopt. One Swedish scientist called the drafts "toothless".

Another senior scientist says he will not attend the Budapest meeting because he is not convinced that his time there would be usefully spent. "I am hesitating in saying this because I know that many people in developing countries have put a lot of effort in preparing for this conference, and rate Unesco highly. But there will be too many people [at the meeting], and too much talk."

Falk says that few will disagree with the aims and objectives of the draft declaration. "But we don't know who will be responsible for implementing the recommendations," he says. The agenda for action, according to Falk, should have clear lines of responsibility.

Falk is also concerned that the conference programme could become dominated by natural scientists, even though Unesco had been presented with a rare opportunity to put together a "forward-looking" programme by bringing together natural scientists with social scientists and academics from the liberal arts.

Full text: <http://helix.nature.com/wcs/a35.html>

Nobel laureates lined up for US delegation

[WASHINGTON] A pair of Nobel laureates, President Bill Clinton's science adviser, the president of the National Academy of Sciences, two immediate past presidents of the American Association for the Advancement of Science (AAAS), and a student from Illinois Math and Science Academy, are among a planned 20-strong US delegation to the World Conference on Science.

The list has been put together by the National Academy of Sciences, the main US contact point with the International Council for Science (ICSU) which is organizing the meeting with Unesco.

Although the members of the delegation have yet to be formally approved by the State Department, academy officials expect little

change to the final list. This will be headed by Bruce Alberts, the president of the academy, and include Nobel laureates F. Sherwood Rowland of the University of California, Irvine, and Leon Lederman, director emeritus of the Fermi National Accelerator Centre. Neal Lane, the president's science adviser and head of the Office of Science and Technology Policy, will be accompanied by two of his staff members.

The delegation will include Jane Lubchenco, AAAS president in 1997, and the AAAS immediate past president M. R. C. Greenwood. Also present will be Maxine Singer, president of the Carnegie Institute of Washington, and Keith Winstein, a student from Illinois.

Full text: <http://helix.nature.com/wcs/a36.html>

Alarm raised over elephant ivory trade

Sir— We wish to express our concern over the resumption of a limited international trade in elephant ivory. The Convention on International Trade in Endangered Species (CITES) has allowed Namibia, Zimbabwe and Botswana to sell 59.8 tons of stockpiled ivory to Japan. The sales began in April, ending a ten-year ban. They are termed 'experimental', suggesting that, if deemed a success, further sales will be proposed.

When these sales were initially approved in June 1997, an important condition was attached — that a system should be developed to measure the impact of trade on elephant populations. The recent authorization reflects the satisfaction of CITES with a draft of a trade and elephant monitoring system called MIKE (Monitoring Illegal Killing of Elephants). We feel that the approval of MIKE was inadvisable. This programme may be able to measure large-scale changes in populations, but it cannot gather the details needed to link subtle changes to causes.

Each of us has attempted to count elephants by various methods, and can attest that a reliable, sensitive census is difficult in the best of circumstances. We believe it would be nearly impossible to devise a method that was sensitive to minor changes in populations in varied environments and capable of relating these changes to underlying causes. At best, this would require measurements not included in MIKE's design. Without a good monitoring programme, the impact of the ivory trade will remain unknown.

We are also uncomfortable with the lack of a requirement for a monitoring system to be up and running before the current ivory trades were made. These omissions, and the notion that these sales are 'experimental', spell a dangerous situation for elephants.

CITES meets next in April 2000 in Nairobi. If further sales are proposed, these points must be made:

1) The lack of a satisfactory draft for a system to monitor the global elephant

population and ivory trade means that there will not be enough information available in 2000 to justify further sales.

2) Any population-monitoring system must be scientifically credible, evidenced by peer review, which MIKE largely lacked when it was accepted by CITES.

3) Without a way of assessing the relationship between trade in ivory and the health of elephant populations, further sales must not be authorized.

This letter does not necessarily reflect the opinions of the writers' supporting institutions.

Katy Payne*, **Iain Douglas-Hamilton†**,
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Guidelines point the way on genetics ethics

Sir— You reported that the World Health Organization (WHO) has published draft guidelines on bioethics (*Nature* **398**, 175 & 179; 1999). I would like to draw attention to an earlier effort completed by WHO in spring 1998 — "Proposed International Guidelines on Ethical Issues in Medical Genetics and Genetic Services". As co-rapporteur of these guidelines, I consider them some of the most comprehensive on ethical issues in clinical genetics, while they also address several research issues. The more recent general guidelines on bioethics reiterate some of their provisions.

WHO began its efforts on ethics and genetics in 1995, and a final document was drafted by 16 WHO experts in 1997. The experts were divided on the issue of human cloning and refrained from making hasty conclusions, preferring to issue a compromise "statement of fact" that cloning "has been rejected by many international bodies" and "is not in accord with currently accepted international standards". Most of those present at the meeting considered cloning to be peripheral to the practice of medical genetics and of minor importance when compared to critical issues of access to services, fairness and education.

Since the proposed guidelines pertained mainly to services, rather than to research

ethics, embryo research and germline gene therapy were not discussed. In general, the group opposed rushing to legislate for these ethical issues.

The proposed genetics guidelines were presented at the World Health Assembly in May 1998 and will be presented again in 1999. I would recommend readers to peruse them, and to comment or suggest revisions (<http://www.who.int/ncd/hgn/hgnethic.htm>).

Dorothy C. Wertz

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Genentech stands by original data

Sir— A trial is under way in San Francisco, California, involving a patent dispute between Genentech, Inc. and the University of California at San Francisco (see page 289 of this issue and ref. 1). During this trial, Peter Seeburg of the Max Planck Institute for Medical Research in Heidelberg, Germany, testified that data presented in a 1979 paper published in *Nature*², of which he was a co-author, were false and that he knew they were false when the paper was submitted for publication.

As the other co-authors of that paper, we, together with Genentech, categorically deny this accusation that the data submitted in the *Nature* paper were false. We also

emphatically disagree with Seeburg's statements, made under oath, that it is permissible to make up data on the basis of "similar work" because "it's all the same game", and that publishing a description of a non-existent plasmid is merely a "technical inaccuracy". We believe that to do as Seeburg suggests is both intellectually dishonest and antithetical to the principles on which scientific enquiry rests.

The trial is about patent infringement. The verdict, and the appeals that will doubtless follow, will be about that issue, not about the accuracy of the scientific conclusions of the paper. Nevertheless, we feel that these accusations have falsely impugned our reputation and cast doubts on our scientific integrity.

Genentech retains the notebooks upon which our 1979 *Nature* paper was based. They can be seen on the web at <http://www.gene.com/notebooks/>. In our view, these notebooks provide documentation that this work was indeed performed as described in the paper. We have invited the editors of *Nature* to examine these materials, and to speak to the co-authors, to satisfy themselves as to the accuracy of the paper. We would welcome such an opportunity to resolve these issues.

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Switzerland

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Statement from Peter Seeburg

Sir—I, as an inventor of a patent held by the University of California on cloned human growth hormone complementary DNA, was a witness in a trial involving a patent dispute between the university and Genentech (see page 289 of this issue and ref. 1).

My testimony concerned events that occurred 20 years ago, my work at the University of California, San Francisco (UCSF) and early work at Genentech, which collectively resulted in the expression of human growth hormone (hGH) in bacteria. This pioneering work with my colleagues at Genentech was the culmination of three years of previous efforts at UCSF by which I and my colleague John Shine had succeeded in cloning the main part of the coding sequence for human growth hormone. It had been a difficult personal time as this project had often involved working nights, owing to efforts by my lab head to stop my research on growth hormone, as documented in the 1987 book *Invisible Frontiers: The Race to Synthesize a Human Gene* by Stephen S. Hall (Tempus Books of Microsoft Press).

As I testified during the trial, the *Nature* paper² reporting the landmark study by Genentech regrettably contains a technical inaccuracy. This inaccuracy concerns a plasmid, pHGH31, which represents one of the intermediate steps in the construction of the expression vector for hGH. In this plasmid, the coding region for amino acids 24–191 plus 3' noncoding sequence, all contained on a 551-base-pair *Hae*III complementary DNA fragment, is inserted by 'GC tailing' into the *Pst*I site of pBR322. Not this plasmid, but an equivalent one carrying the same 551-base-pair *Hae*III fragment inserted by linkers in the *Hind*III site of pBR322 and previously constructed by me and Shine at UCSF, was used as source of the natural coding region for amino acids 24–191 in the construction of the final hGH expression vector.

The existence of pHGH31 is questioned

by the fact, acknowledged by Genentech, that there never was a sequence record showing hGH DNA sequence attached to a G or a C tail, even though such a record should have existed, according to the *Nature* paper. Several attempts at Genentech by a colleague and me to obtain pHGH31 were unsuccessful, primarily due to the poor quality of the RNA starting material available to us at the time. With increasing pressure to complete the expression work, my colleague and I agreed to use the University of California's cDNA clone for part of the work.

To be absolutely clear, I, like my coauthors, view it as mandatory that publications are correct in all aspects, including all technical and methodological details. Hence, I deeply regret that, contrary to my own principles and the principles of scientific endeavour, the *Nature* paper contains a technical inaccuracy.

As I emphasized during the trial, all scientific results and conclusions of the *Nature* paper are unambiguous and correct. The expression vector is exactly as described and the bacteria make the correct hormone at the levels described in the publication. The study reported in the paper forms the basis for the first human growth hormone preparation free of neurodegenerative agents³, and the first recombinant therapeutic to be marketed by Genentech, from which 100,000 children benefit worldwide.

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1. Marshall, E. *Science* **284**, 883–886 (1999).

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Innocents suffer as rogue regime rapped

Sir—Christian Seelos's Commentary, "Lessons from Iraq on bioweapons", discussed only active warfare, where the weapons themselves contain infectious materials or biological toxins (*Nature* **398**, 187–188; 1999). He failed to mention an equally nefarious kind of biological warfare, of the passive variety.

The bombing of Iraq during the Gulf War targeted infrastructure, with drastic effects on public health. One of the many results was a lack of water free of infectious particles, which led to a resurgence of bacterial infections, especially infantile diarrhoea, cholera and many other infectious diseases. The mortality rate for infants soared, with excess mortality of

close to a million children, exacerbated no doubt by the severe malnutrition that the United Nations embargo has imposed.

One factor that exacerbated this problem was the lack of chlorine, which the UN Special Commission (UNSCOM) has decreed to be, in Seelos's sanitized phrase, "dual use". Eventually another UN agency, the children's fund UNICEF, campaigned to allow chlorine back, but the amount recently allowed is probably enough for only two or three cities. This kind of biological warfare is similar to poisoning wells or rivers upstream of besieged cities, which has its own long and notorious history.

Yet these issues are not discussed, perhaps because the personal viewpoint of Seelos, or the official one of UNSCOM, is that only when you lob the carcass of an infected animal into a besieged city do you commit the horrible crime of biological warfare. Or else, they might simply state, in the words made famous 50 years ago, that they were only following orders.

How many more people have to die before we decide that the price of our policies towards the rogue regime of the day is unsupportable?

Qais Al-Awqati

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Science powerhouse of Central America

Sir—Small is beautiful, but when you are small nobody sees you. Democratization of Latin America is our chance to progress as a region, as your supplement on *Science in Latin America* demonstrates (*Nature* **398** (Suppl. 1 April); 1999). The articles on recent developments in Mexico, Chile, Argentina, Brazil and Cuba are inspiring. But only the largest countries of the region were considered. There are also small countries that have scientists who are trying to make a difference.

Costa Rica has a population of 3.5 million and a research budget much smaller than the US\$108 million indicated in the figure on page A5 of the supplement. Yet it produces more scientific papers than much larger and richer countries. It has been an uphill challenge for us, however. The government is unsupportive of science, and we suffer all the maladies described in the supplement.

Even so, Costa Rica has managed to be the science powerhouse of Central America, and we will continue our fight to advance science in this minuscule country.

Jorge Cortés

CIMAR, Universidad de Costa Rica,
San Pedro, Costa Rica

Will biomedicine outgrow support?

The number of scientists in the biomedical field is growing exponentially at rates that outstrip funding. The present system of short-term research grants, resulting in armies of postdocs without career prospects, must be changed.

M. F. Perutz

Throughout the developed countries, biomedical science has grown spectacularly in the past 50 years. The benefits to society, and the fascination of understanding how life works, have attracted growing numbers of young scientists and medical graduates to biological research. Support by governments and charities has also grown, but nevertheless, scientists in both Europe and the United States suffer from a funding crisis that has made it increasingly hard to obtain support. For this reason, and out of curiosity, I have estimated the rise in the numbers of scientists engaged in the field since the Second World War, and compared this rise with the rise in the amounts of government money spent on it in the United States and in two representative European countries:

Britain and Germany. Below I describe my unexpected findings and discuss their implications for the future development of the biomedical sciences and of their industrial applications in the United States and Europe.

Growth in numbers

A rough measure of the increase in the number of scientists engaged in a particular field can be obtained from the registers of members of scientific societies. FASEB (the Federation of American Societies for Experimental Biology) issues an annual directory of 14 societies, ranging from anatomy to biophysics. A plot of the logarithm of the number of entries against time gives a straight line, with a constant annual growth rate of 6.4% and a doubling time of 11 years, from 469 in 1920 to 56,469 in 1997–98 (Fig. 1).

This growth rate is even faster than that of 5% in the number of PhDs recorded in the recent report *Trends in the Early Careers of Life Scientists* by the National Research Council (NRC; see *Nature* 395, 103; 1998). From the mid-1930s until about 1970, the American Physical and Chemical Societies showed growth rates similar to those of the biological societies, but since then they have slowed to 2.6 and 1.8%, respectively.

European trends are harder to measure, because there are no organizations comparable to FASEB. From 1920 to 1970, the British Biochemical Society grew at an average annual rate of 5.8%; from 1950 to 1970 the Gesellschaft für Biochemie und Molekularbiologie grew at an annual rate of 16%. After 1970, their rates of growth slowed to 2.4% and 10%, respectively; the societies now have memberships of around 10,000 and 5,000, respectively. The growth rate of the German society outstrips that of FASEB, probably because it started later.

These rates are reflected by the rise in the number of scientists working at three of the laboratories most active in the field: the Cold Spring Harbor Laboratory (CSHL), the European Laboratory of Molecular Biology (EMBL) and the Medical Research Council (MRC) Laboratory of Molecular Biology (LMB) in Cambridge, United Kingdom (see Fig. 2, overleaf).

Rise in costs

The most extensive data on costs are available from the UK MRC. Figure 3 (overleaf) shows the costs per member of scientific staff and per bench worker (that is, scientific and technical staff) from 1950 to today, in sterling at current and constant prices, and also the cost per bench worker as a fraction of gross domestic product (GDP). Costs include the total expenditure of the MRC, including its administration, new buildings and equipment. Corrected for inflation, the costs per scientist and bench worker have risen at about the same rate as GDP (2.5%), so that, despite the great increase in sophistication of research, the cost per bench worker divided by GDP has remained constant since 1950. At CSHL and EMBL, absolute costs per scientist have actually fallen. Present annual costs per scientist at 1998 prices are about \$150,000 at LMB and \$200,000 at CSHL and EMBL.

Government expenditure

Government expenditure on biomedical research rose very steeply from 1950 to 1970 in the United States and less steeply in the

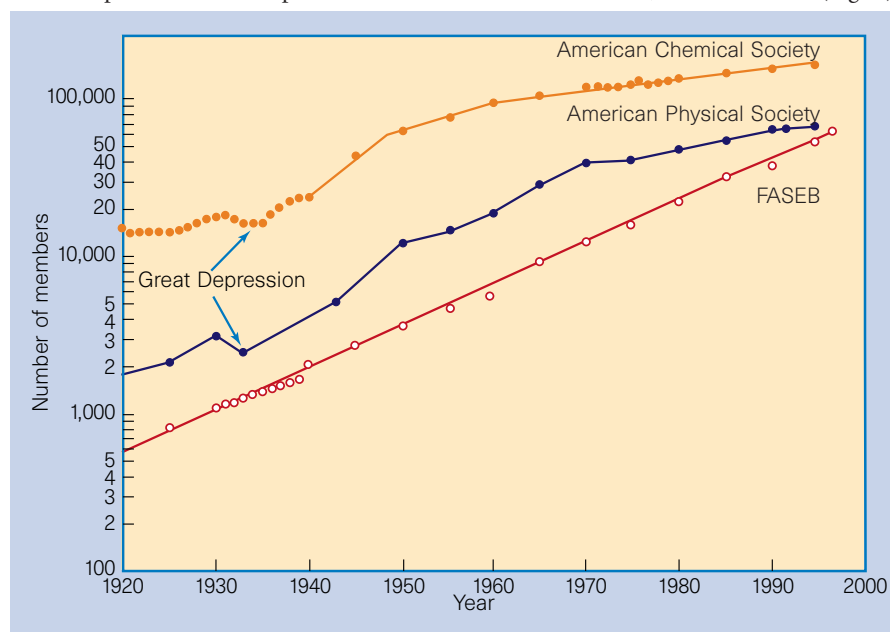


Figure 1 Semilogarithmic plot of membership of some American societies during this century. The curve for the American Physical Society also includes the Optical Society of America, the American Astronomical Society and the American Association of Physicists in Medicine.

Table 1 Support for biomedical research in 1994–95

	Government sources only			Government, charities and industry combined	
	US	UK	Germany	US	UK
Total support (\$million)	15,846*	1,733†	2,676‡	34,600	2,618
Fraction of GDP spent (per cent)	0.0024	0.0016	0.0014	0.005	0.0024
Expenditure per capita (\$)	61	29	33	133	44

GDP was \$6,650, \$1,066 and \$1,858 billion in the United States, United Kingdom and Germany, respectively.

*Includes government, federal, NIH, state and local support.

†Includes the National Health Service, MRC, the Higher Education Funding Councils, the Biotechnology and Biological Sciences Research Council and the Department of Health (data supplied by the Royal Society).

‡Includes total public-health spending, including federal government, Länder and university (data supplied by the Max-Planck Gesellschaft).

United Kingdom and Germany. After the economic crisis in 1970 it levelled off, most dramatically in the United Kingdom, less in Germany and least in the United States (Fig. 4). As a fraction of GDP, it has remained constant in the United Kingdom and Germany, while still growing in the United States. As a result, the number of recipients of grants from the National Institutes of Health (NIH) has risen steadily since 1970, while the number of staff at the MRC and the Max-Planck Gesellschaft (MPG) has remained constant (the latter showed a small rise after German reunification) (Fig. 5).

Table 1 shows the most recent figures available for expenditure on biomedical research in the three countries. In the United States, government expenditure alone was nine times that in Britain and six times that in Germany. As a fraction of GDP, the United States spent 1.5 times as much as Britain and 1.7 times as much as Germany. Per capita expenditure was twice that in the two European countries. This disparity increases when support from charities and the pharmaceutical industry is included (Table 1). It will become even greater in the light of the 15% annual rise in support by the NIH for this year, which is planned to double in 10 years. (Japan, South Korea and Singapore have decided on similar increases, whereas Britain has announced an annual rise of only 2.3% in real terms for the MRC over the next three years.)

Are there too many scientists?

Suppose 40,000 FASEB members are engaged in research. At an annual cost of \$200,000 per worker, the bill comes to \$10 billion. At the present annual growth rate of 6.4% per annum, these numbers will have swollen to 160,000 by 2020, at an annual cost of \$32 billion (1998 dollars). On the other hand, even at an optimistic average annual growth rate of 3.5%, GDP will have only doubled. During the past few years, the NIH budget has risen annually by 3.2% and the numbers of grant recipients by 1.4%. The growth of the MPG has been similar, but that of the MRC much slower, and the number of scientific posts has not increased significantly since 1970, while the number of tenured university posts in Britain has diminished.

By attracting very many young scientists into the biomedical field and by their optimism about its future, all three countries are now faced with large armies of postdocs with poor career prospects. The recent NRC report drew attention to the plight of these people by predicting that even the projected increases in US government funding are unlikely to produce any mitigating growth in permanent jobs, nor was industry likely to

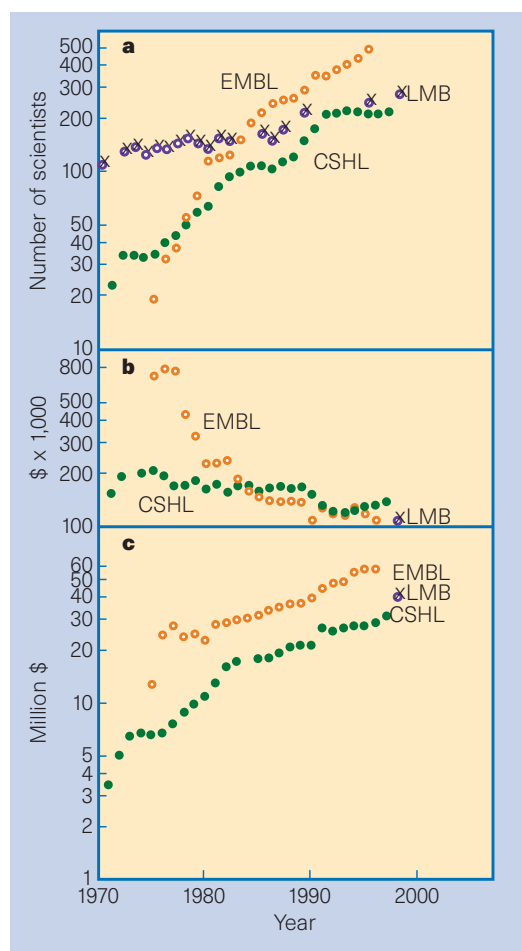


Figure 2 a, Number of scientists at work at the European Molecular Biology Laboratory (EMBL) in Heidelberg, with branches in Hamburg, Grenoble and Cambridge; the Cold Spring Harbor Laboratory on Long Island (CSHL), New York, and the MRC Laboratory of Molecular Biology (LMB) in Cambridge. b, Cost per scientist (in 1990 dollars); c, total costs of these laboratories (in 1990 dollars).

hire more people, given recent trends (see *Nature* 395, 103; 1998). The same probably applies to Britain and Germany. The MRC has to reject a large fraction of first-class grant applications for lack of funds.

The disparity between the United States and Europe in government-sponsored

research is reflected in its industrial applications. In 1997, revenue from sales of biotechnology products for the whole of Europe combined was only one-eighth of that of the United States, and industrial investment only between a third and a half*.

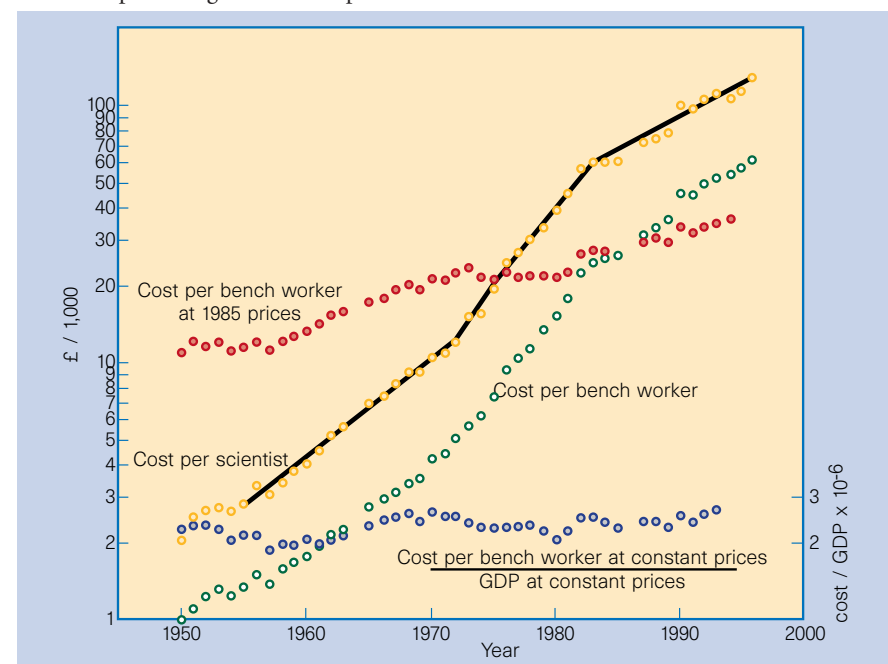


Figure 3 Costs per scientist and per bench worker (scientists and technical staff) employed by the UK MRC.

* Ernst & Young, Inc. 12th Biotechnology Industry Annual Report: New Directions 1998 and European Life Sciences 1998: Continental Shift. 5th Industry Annual Report.

The exponential growth in the number of biomedical scientists, especially that of postdocs on short-term appointments, far outstrips the expansion of government support everywhere. It is clearly at the root of the funding crisis, which has made the search for grants so competitive: writing applications has become a full-time occupation for senior scientists. Success needs publications and publications need postdocs, many of whom aspire to direct their own postdocs one day, so that exponential

growth in numbers is built inexorably into the present system. On the other hand, the vastly expanded American medical schools are in danger of losing their income from patient care through competition from non-profit health-care concerns that do not have to pay for research, so medical schools will be able to take on fewer scientists. Clinical research already suffers in this way.

Will the fast-growing pharmaceutical and biotechnology industry absorb the fast-

growing numbers of biomedical scientists? Its total expenditure on research and development in 1998 has been estimated at \$8.9 billion in the United States and \$2 billion in the whole of Europe. Between 1997 and 1998, that expenditure grew by 12.5% in the United States and by a spectacular 38% in Europe. These figures suggest that the NRC report may have been too pessimistic in its estimates of new job opportunities in industry, although most of them may be for short-term positions.

The grant system needs reform. Too many agencies give short-term grants for postdoctoral assistants without any thought about their future. It would be more humane if they followed the example of the MRC, which selects candidates for appointments with great care and, having appointed them, offers them a career structure. A system of long-term grants would provide fewer openings, but give greater security and would free senior scientists for research, rather than tying them to their desks writing grant applications. The NRC report proposes a restriction on the number of PhD candidates admitted to universities, but such restrictions could not be enforced. If career prospects are not improved, student numbers in the life sciences may fall as they have already done disastrously in chemistry in the United States, and in several European countries when the large chemical firms stopped recruiting.

Improving European competitiveness

What can European scientists do to hold their own better in the future? It may help if they made political, financial and industrial leaders aware of the 1.5–2-fold fraction of GDP devoted to medical research and its applications in the United States. They need to show that this is not only a question of national wealth, but also of political will, industrial foresight and optimism about potential benefits. The European output of biotechnology products is a small fraction of the US output, but this situation could be changed with great benefit to Europe.

The past 50 years of biomedical research have brought spectacular improvements in public health and in the average life expectancy. If the next 50 years can match only half of this, then the large sums of money likely to be spent on that research will be worthwhile. □

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Acknowledgements: I thank the following people for statistical data on the various organizations: David Smith, MRC; Hubert Markl, Max-Planck Gesellschaft; Harold Varmus, NIH; Rose Grimm, FASEB; T. D. Inch, Royal Society of Chemistry; Philip Diamond, Institute of Physics; M. Bowen, American Chemical Society; Roman Czujko, American Institute of Physics; James Watson, CSHL; Mary Holmes, EMBL; Heinrich von Dieck, Gesellschaft Deutscher Chemiker; and Heinz Metzger, Gesellschaft für Biochemie und Molekularbiologie. I thank S. Solomou for advice on economics.

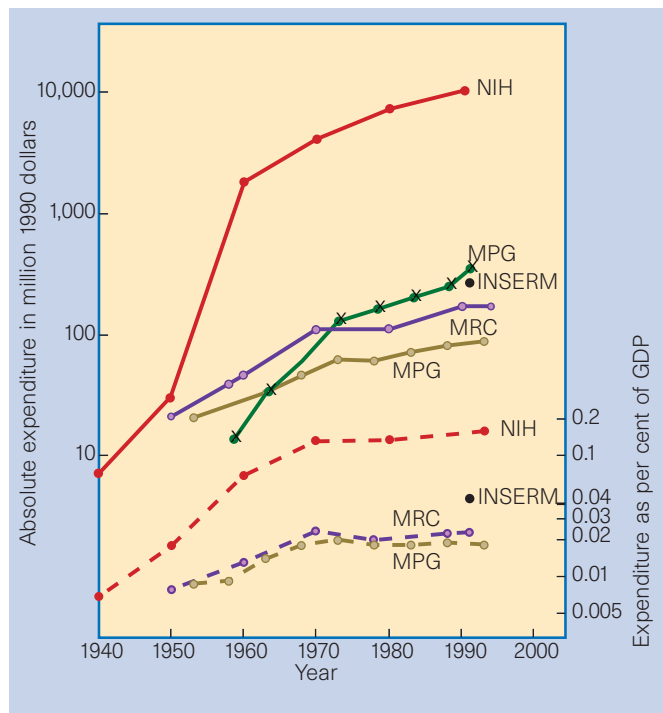


Figure 4 Government expenditure on medical research in the United States, Britain and Germany from 1950. The points marked INSERM refer to the Institut National de la Santé et de la Recherche Médicale in France (figures not available for other years).

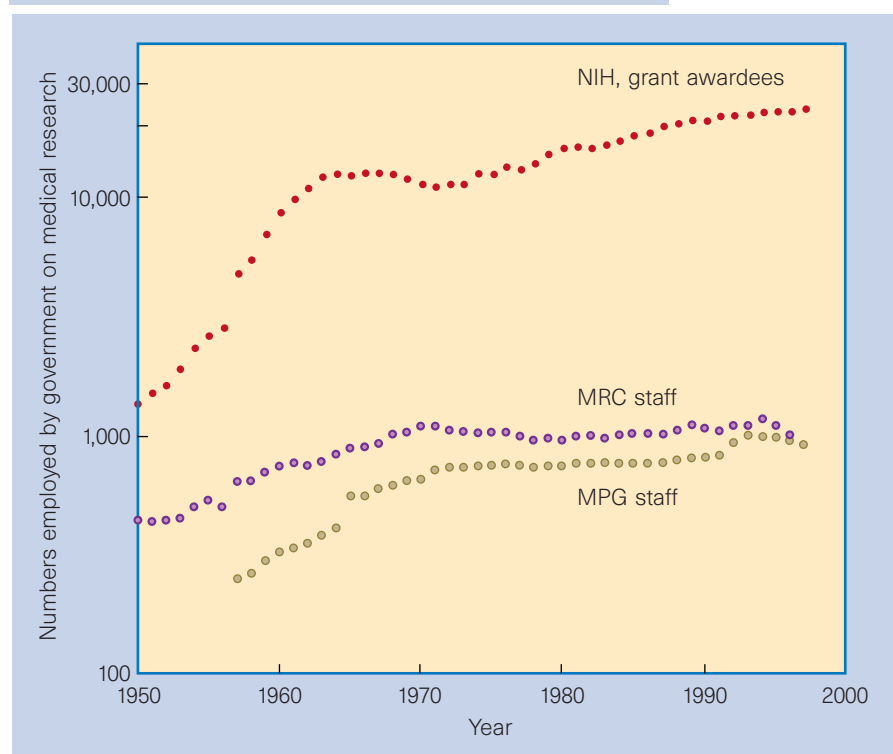


Figure 5 Growth in numbers of NIH grant awardees and of the scientific staff of the MRC and the MPG.

Reinventing the engine

Steven L. Garrett

Glass-blowers have known for centuries that a heated vessel can generate sound. Thermoacoustic energy conversion can now be made as efficient as energy conversion in an internal-combustion engine – a feat that has been accomplished in a device with no moving parts.

The operation of the internal-combustion engine depends on the proper phasing of the compression, ignition, expansion and exhaust stages of the power cycle. This phasing is maintained by mechanical means. Modern car engines may require as many as 24 valves (each with a rocker arm, spring, push rod, cam and so on) to produce the required phasing. The thermoacoustic Stirling heat engine, described by Backhaus and Swift on page 335 of this issue¹, has a thermal efficiency (ratio of net work produced to fuel energy used) of 30%, comparable to that of a car engine (25–40%). They use carefully shaped acoustic ducts to provide the required phasing, thereby eliminating any moving parts, other than the acoustic oscillations of the gas. There are no sliding seals or machined parts requiring tight tolerances and lubrication. The working fluid is pressurized helium, an inert gas, which neither depletes stratospheric ozone nor contributes to global warming. The simplicity and efficiency of their thermoacoustic approach could provide new, environmentally friendly options to industries as diverse as transportation, natural-gas liquefaction, refrigeration and air conditioning (see Box 1, overleaf).

Over the past two decades, substantial progress has been made in thermoacoustic engine and refrigerator development. Thermoacoustic devices have flown on the space shuttle², cooled radar electronics on a US Navy destroyer³, and liquefied 140 gallons of natural gas per day⁴. In these earlier devices, the gas used as the working fluid is placed in thermal contact with a solid medium, called a 'stack' owing to its resemblance to a pile of plates. During periodic fluctuations in gas pressure, the gas passing through the stack in an engine is heated at the proper phase in the acoustic cycle to amplify the oscillations. This is similar to the way light waves are amplified as they reverberate within the optical resonator of a laser.

The gas-filled interstices within these stacks are comparable in size (~ 0.5 mm) to the distance over which heat can diffuse during one-quarter of the acoustic cycle — typically about a millisecond. The imperfect thermal contact in the stack's pores provides the phasing between the compressions,

expansions and acoustic displacements necessary to lead the gas through the desired thermoacoustic cycle. The phasing for this cycle is almost the same as the phasing of an acoustic standing wave within a confined space, such as the wave motion of air in an organ pipe. The pressure fluctuations lead or lag the gas velocity oscillations by nearly a quarter of a cycle. This produces very simple engines and refrigerators, but the entropy generated by the irreversible thermal transfer between the gas and the stack limits the efficiency of such 'standing-wave' thermoacoustic engines. In the late 1970s, Peter

Ceperley⁵ anticipated that higher efficiencies could be obtained using a solid medium with much smaller pore sizes — a Stirling-cycle 'regenerator' rather than a 'stack'.

The history of the Stirling cycle goes back to 1816, when the Reverend Robert Stirling invented a hot-air engine that operated without a high-pressure steam boiler. The Stirling engine depended for its power on the expansion and displacement of air inside a cylinder, heated by external combustion through a heat exchanger. Stirling also conceived the idea of a regenerator to store thermal energy during part of the cycle and return it later. This increased the efficiency of the device, but its mechanical complexity was greater than internal combustion engines (which do not require heat exchangers), resulting in high production costs and restricting its widespread use.

Ceperley recognized that the phasing in a Stirling-cycle engine was essentially the same as in a travelling acoustic wave — pressure and velocity are in phase. The gas-filled pore sizes in the regenerator are so small that the high heat capacity of the regenerator material (usually tightly packed metal screens) causes the gas temperature to be the same as that of the regenerator at all times. But there

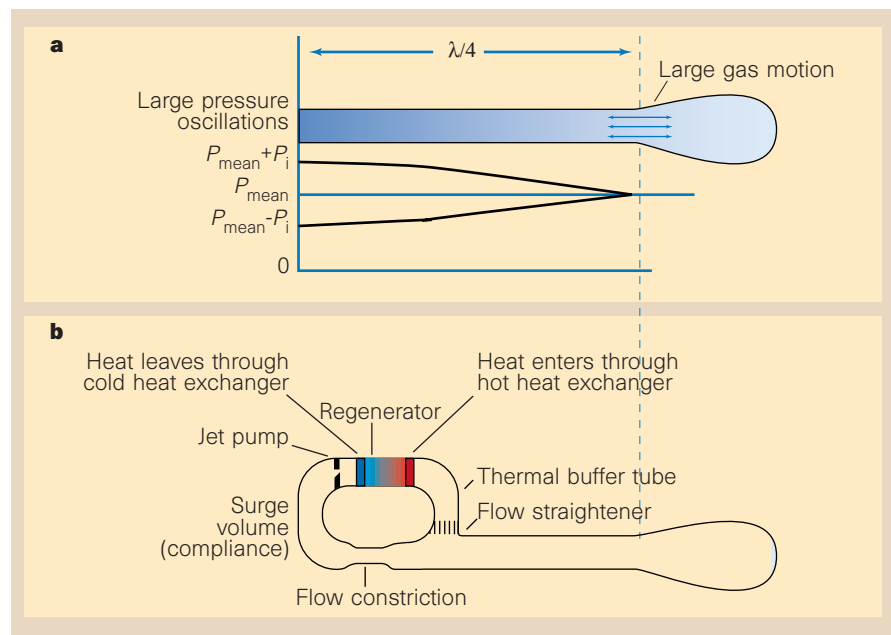


Figure 1 The workings of the thermoacoustic Stirling engine. **a**, A quarter-wavelength standing-wave resonator similar to that used by Backhaus and Swift¹. The bulb is able to store gas and reduce pressure oscillations at the 'open' end of the resonator, making it half as long as it would have been if both ends were 'closed'. As the gas sloshes towards the closed end of the tube, the pressure increases above the mean pressure. As the gas is forced back into the bulb by the excess pressure at the closed end, the gas overshoots its equilibrium position and the pressure at the closed end is reduced below the mean pressure value. This oscillation of the gas in and out of the bulb occurs at the lowest natural resonance frequency. **b**, Backhaus and Swift exploit the large pressure oscillations at the closed end of the tube to force gas through the regenerator in a Stirling-cycle engine. They produce a pressure difference across the regenerator by adding a gentle flow constriction and some extra volume that induces gas flow velocities through the regenerator with travelling-wave phasing. The tapered orifice behind the cold exchanger acts as a 'jet pump' to suppress streaming flow around the loop. The flow straightener keeps the gas in the thermal buffer tube (shown here as bent, but actually straight and slightly tapered) from being stirred up by the turbulence at the junction of the loop and resonator.

Box 1: Cool sounds and hot loudspeakers

Like many other heat engines, the operation of a thermoacoustic engine can be reversed. Instead of supplying heat and generating sound, as demonstrated by Backhaus and Swift¹, mechanical energy could be supplied in the form of sound. This would mean the engine could be used to pump heat from a low temperature to a higher temperature, thus producing useful refrigeration. Several devices that incorporate both a thermoacoustic engine to generate sound, and harness that sound to produce thermoacoustic refrigeration, were developed at Los Alamos⁷ and elsewhere¹¹ to create heat-driven refrigerators, again without requiring any moving parts.

Most refrigerators and air conditioners use electrical energy, not heat. To exploit thermoacoustic-refrigeration technology in those applications, a loudspeaker is required to convert electrical power into high-intensity sound. This electroacoustic energy conversion must be accomplished at high



efficiency so that the overall efficiency of the refrigerator or air conditioner is not compromised.

Speakers used in home stereo systems or in theatres must cover the entire audio spectrum (20 Hz to 20 kHz), but they rarely produce more than a few watts of radiated acoustic power or achieve electroacoustic conversion efficiencies in excess of 1%. Recent advances in linear motors¹² have made possible the development of loudspeakers with limited frequency ranges, but which can produce kilowatts of acoustic power with measured efficiencies as high as 85% (ref. 9).

A high-power, high-efficiency loudspeaker⁹ developed for a shipboard thermoacoustic air conditioner with a 10 kW cooling capacity is shown here (patent pending). Unlike conventional

loudspeakers, which use a moving coil attached to a felt cone to radiate sound, this loudspeaker uses a stationary coil and magnets that are attached to a moving piston¹². The felt cone is replaced by a metal bellows that can withstand large pressures and still provide a flexible seal that has low loss and can operate for 100 billion cycles.

The acoustic amplitudes within these thermoacoustic refrigerators and engines reach sound levels around 190 dB. That is about ten million times as intense as the front-row levels at a rock concert and 300 times the intensity required to ignite human hair! But because the sound is produced inside a rigid pressure vessel, the sound levels outside these devices are much lower, and can be lower than existing vapour-compression systems of similar cooling capacity. In applications that require low sound levels, it is easy to cancel the residual sound electronically because only a single-frequency tone is radiated. **S. L. G.**

were problems with Ceperley's engine that prevented it from reaching its potential efficiency. The small pore size produces significant frictional losses as the gas flows through the regenerator. Additionally, the toroidal geometry proposed by Ceperley allowed the gas to stream between the hot and cold heat exchangers, wasting a large fraction of the engine's thermal energy. These limitations delayed the fabrication of any useful engines based on the travelling-wave concept.

Backhaus and Swift¹ now provide elegant acoustic solutions to both of these problems in their thermoacoustic Stirling engine, developed at Los Alamos National Laboratory. They placed their regenerator in a loop near to the closed end of their acoustic standing-wave resonator. At this end of the resonator, the gas motion is small, because it

cannot penetrate the closed end, but the pressure swings can be very large at the resonance frequency (Fig. 1a). During one half of the acoustic cycle, the pressure is increased by the motion of the gas rushing from the 'open' bulb of the resonator towards the closed end. The increased gas pressure eventually stops the inflow and acts as a spring that accelerates the gas away from the closed end during the next half of the acoustic cycle. Because of its inertia the gas overshoots its equilibrium position, pulling gas away from the closed end and creating a pressure minimum. At the closed end, the ratio of pressure to volumetric flow rate (known as the acoustic impedance) in the Backhaus–Swift engine is 30 times larger than that of an acoustic travelling wave, thereby reducing the frictional losses within the regenerator.

A clever arrangement involving a surge volume (compliance) and a gentle flow constriction (feedback inertance) near the closed end of the resonator (Fig. 1b) produces sufficient pressure difference across the regenerator to force the gas through it with the proper travelling-wave phasing. The combination of travelling-wave phasing and high acoustic impedance allows the stack to be replaced by a regenerator, which nearly doubles the efficiency of previous stack-based thermoacoustic engines⁶.

A further problem is created by the large acoustic power flow in the engine. This causes streaming of the gas, which can 'short circuit' the hot and cold ends of the regenerator and waste large amounts of heat. A novel 'jet pump' is incorporated in the Los Alamos engine, which uses acoustics to create the back pressure necessary to cancel the streaming. The jet pump relies on the asymmetry between inflow and outflow through a tapered orifice. In oscillatory (acoustic) flows, summing these flow-direction-dependent pressures results in a net pressure at the orifice⁷, which Backhaus and Swift adjusted to suppress the streaming.

Thermoacoustic engines and refrigerators were already becoming attractive alternatives in specialized applications^{2–4}, where their simplicity, lack of lubrication or sliding seals, and their use of environmentally harmless working fluids, have been adequate compensation for their lower efficiency. This latest breakthrough¹, coupled with the earlier development of flow-through thermoacoustic refrigerators⁸ and the demonstration of high-power, high-efficiency loudspeakers⁹ (see Box 1), should eliminate the current efficiency deficit.

There is little doubt that this emerging technology, invented by a small cadre of physicists, will be further improved when the technical talents and resources of the engineering community are applied to the development of new thermoacoustic products. Much needs to be done to exploit this technology for applications that need mechanical or electrical energy. For example, the acoustic power generated by the Backhaus–Swift engine could be used to drive a loudspeaker backwards. The pressure on a moving piston could move the magnets back and forth through the coils and generate electricity to recharge batteries in an electric vehicle.

Thermoacoustics may soon emerge as an environmentally attractive option for powering hybrid electrical vehicles, solar energy conversion¹⁰, refrigeration, air conditioning and potential large-scale applications yet to be imagined. □

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Ecology

When one eye is better than two

Mandyam V. Srinivasan

Evolution has generated a bewildering variety of life forms on this planet. Curiously, it has also occasionally produced animals from entirely different families that seem to have converged on a similar design plan — apparently as a result of similar lifestyles and environmental pressures. One instance of this, reported by Pettigrew *et al.*¹ and Fritsches and Marshall² in *Current Biology*, is vision in the chameleon and in the sandlance *Limnichthys fasciatus*, a small teleost fish^{1–4}.

The chameleon catches insects with a quick flick of its long tongue⁵. The sandlance lies concealed in underwater sand beds with only its eyes protruding above the surface, then lunges at tiny, unsuspecting copepods (aquatic crustaceans) that stray too near⁴. Pettigrew *et al.* and Fritsches and Marshall now show that there are extraordinary parallels between these two behaviours, and between the visual systems that mediate them.

To a casual observer, the dart of the chameleon's tongue is very similar in its trajectory to the lunge of the sandlance. The sandlance is, in effect, a chameleon's tongue with eyes at the tip¹. Moreover, both the chameleon and the sandlance move the two eyes independently and alternately (Fig. 1)^{1,2} — while one eye remains motionless, the other scans the environment in a saccadic (jerky) fashion, presumably seeking prey. The two eyes alternate when looking around the environment, and each covers about half the panorama. Scanning alternately with the two eyes reduces the chance of alerting the prey by tell-tale eye movements. Plus, given that the two eyes are not yoked together to look in the same direction, it would be sensible not to move them simultaneously. Otherwise, it could be difficult for the animal to decide whether the two eyes were viewing the same target.

In both animals, the cornea of the eye — rather than the lens — provides the bulk of the refracting power. This extends the effective focal length of the eye, giving it a higher angular resolution¹. Moreover, the cornea (not the lens) enables the eye to change its focus and view objects at different distances. This property makes the sandlance very



Figure 1 Keeping an eye on things — independent eye movements in the chameleon and sandlance. The activity of the two eyes alternates, each covering about half the panorama. Pettigrew *et al.*¹ and Fritsches and Marshall² show that there are many other parallels between the visual systems of these two animals, which are surprising given that they come from such evolutionarily distinct families.

different from most other fish, which focus mainly through the lens³. Pettigrew *et al.*¹ show that, in both the sandlance and the chameleon, the refracting power of the cornea is adjusted by a fast-acting, striated 'cornealis' muscle. There is also evidence that the chameleon perceives depth by monitoring how much one eye is focused, rather than by combining the images from both eyes (stereopsis)⁵. The sandlance probably adopts a similar tactic¹, meaning that both animals

can, and do, strike at a target using visual information from just one eye.

The stronger refraction provided by the cornea (as opposed to the lens) means that, in each animal, the nodal point — the point within the eye at which the lines connecting points in the scene and corresponding points in the image intersect — lies well in front of the centre about which the eye rotates^{1,6}. As a result, when the eye rotates, the images of objects at different distances move by different amounts on the retina (Fig. 2). This means that rotation of a single eye can provide information about the relative distances of different objects. The animal can even estimate absolute distances if the amount of eye rotation is known. By gauging the distances of objects by rotating just one eye, without using binocular stereopsis, and without having to move the whole head as some insects do^{7,8}, the chameleon and the sandlance minimize their chances of being detected by potential prey^{1,3}. At present, however, such a means of distance estimation by these animals is only a theoretical possibility.

Despite belonging to such different families, the chameleon (a reptile) and the sandlance (a fish) seem to have converged on a common set of design principles for their visual systems. So, in these two animals, eye design cannot be predicted by evolutionary origin — rather, it has been crafted almost exclusively by environmental constraints. But the visual system of the sandlance seems to differ from that of most other vertebrates (including the chameleon) in that, after the eye has moved to a particular position, it does not stay there. Instead, Fritsches and Marshall² find that the gaze drifts slowly back towards the central viewing direction, presumably because of restoring forces exerted by the muscles that rotate the eye. This drift challenges the accepted idea^{3,9} that animals strive to keep a still image of the world on

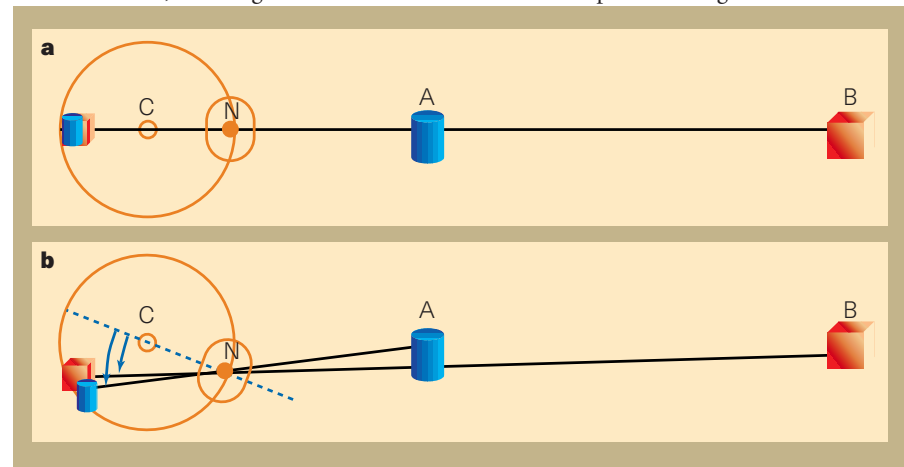


Figure 2 How the sandlance and the chameleon could gauge depth by rotating a single eye. a, When the eye faces straight ahead, objects A and B are imaged at the same place on the retina. b, When the eye rotates, however, the image of A moves by a larger amount than that of B, revealing that A is closer than B. This method of estimating distance is possible only if the nodal point of the eye (N) is located well in front of the centre of rotation of the eye (C), as is the case in these animals.

their retina most of the time, to minimize blur and to detect stationary or moving objects more easily.

So why does the sandlance's gaze drift? One possibility is that there is simply not enough visual texture in the murky, underwater world in which it lives to help stabilize the eye^{2,3}. Another intriguing idea is that the slow drift is exploited by the sandlance to infer the distances to objects (Fig. 2). But, as Fritsches and Marshall point out, another advantage in drifting back to the central position is that the eye is then best positioned to move to the next target of interest, which may appear anywhere in the visual field. This strategy is akin to that used by tennis players, who return to mid-court after dispatching each ball. Indeed, the sandlance eye may

be a drifter with a purpose, and not just an aimless wanderer. □

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Planetary science

Plate tectonics on Mars?

Dan McKenzie

Two papers in *Science*^{1,2} report the first observations of martian magnetic anomalies, made with magnetometers on board the Mars Global Surveyor spacecraft. This discovery was completely unexpected. Some of the anomalies are large (about 1,000 nanotesla) and well resolved, and they are mainly in the older, heavily cratered southern part of the planet¹.

Although the satellite tracks are rather widely spaced, some of the anomalies are clearly lined in an east–west direction (Fig. 1). In consequence, Connerney *et al.*² argue that they may have been produced by plate motions early in martian history. The suggestion that plate tectonics has operated on a planet other than the Earth is of great interest, because we still can only speculate on why the process has been so important on this planet, but not on Venus, which is otherwise so similar.

Before the 1960s, several Earth scientists had suggested that continental drift had occurred on Earth, but most found the evidence unconvincing. What changed everyone's mind was the discovery of linear magnetic anomalies, first in the northeast Pacific, and then in all of the world's oceans. When they were discovered these anomalies were very puzzling. They are linear for long distances, but are sometimes interrupted by large faults which offset the entire pattern of anomalies by as much as 1,000 km. The anomalies themselves have characteristic profiles that can easily be recognized wherever they occur.

An explanation was proposed by Vine and Matthews³ in 1963. They had carried out a survey of an underwater volcano near the Seychelles, and found that it was formed when the main magnetic field of the Earth was in the opposite direction to what it is now.

That the Earth's field reverses was already known then, but our understanding of why it should do so remains poor. The field is produced by dynamo action, caused by fluid movements in the Earth's liquid-iron core. The governing equations remain unchanged if the sign of the magnetic field is reversed. So a given flow field can support a magnetic field in either direction, but we don't know how to calculate the strength of the field or the frequency of the reversals. Although the core

must be fluid to act as a dynamo, the reverse is not true: Venus has a fluid core but no dynamo. What Vine and Matthews realized was that such reversals could account for the linear magnetic anomalies on the sea floor if new oceanic crust is produced by volcanism along a line forming the boundary between two plates that are separating. As the molten rock formed by this separation comes into contact with sea water, it cools and becomes magnetized by the Earth's magnetic field. When the field reverses, so does the direction of magnetization, producing long strips of oceanic crust magnetized either along the present magnetic field or in the opposite direction. It is these strips that give rise to the magnetic anomalies.

On Earth, the anomalies have allowed us to reconstruct plate motions in great detail, but we are very lucky to be able to do so. The reversals are infrequent and aperiodic: if they occurred more often, every few hundred years or so, they would not produce strips of magnetized crust, because the volcanism at plate boundaries is spread over a few kilometres and the pattern would be too smeared out to be visible. Furthermore, if the reversals were periodic, like those of the Sun, which reverses its magnetic field every 11 years, all of the anomalies would look the same, and we would not be able to use them to date the sea floor.

Another piece of luck is that the ocean basins are full of water, which rapidly cools the molten rock and so stops it from flowing far from the plate boundary; rapid cooling

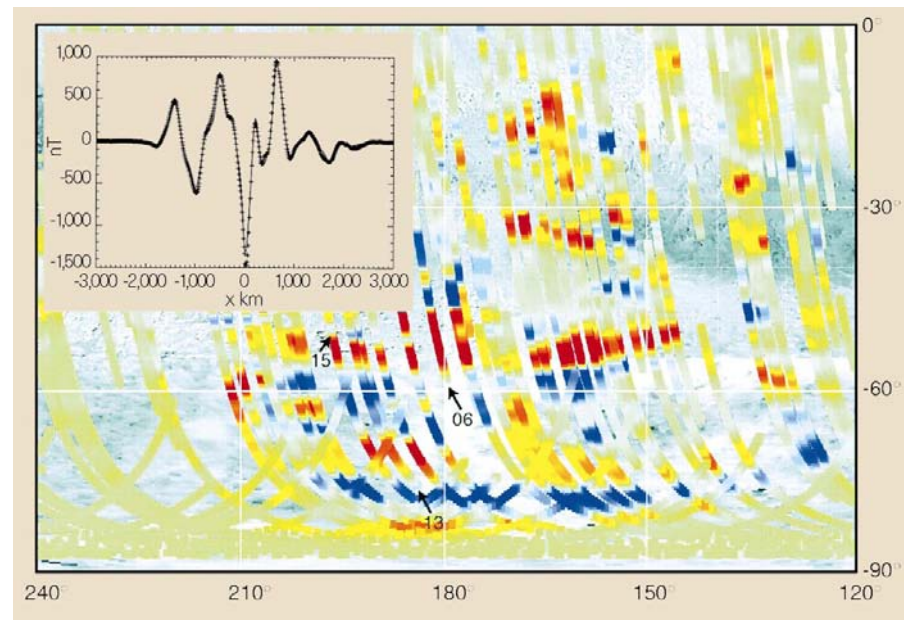


Figure 1 Map of the martian magnetic field, from ref. 2. Each curved strip shows the field along a spacecraft track where its height is less than 200 km, with red and blue colours showing where the radial field is positive and negative. Notice the correlation between adjacent tracks, even though the spacecraft height varies, and the absence of features near the equator, where the crust is younger¹. The inset shows a profile of the vertical magnetic field (positive downwards) from the pass marked 15 on the map, plotted against distance from the origin at 53° S, with north as positive. The field variations are very large compared with the largest on Earth, which are of a few tens of nanotesla at these heights.

also produces very fine-grained basalt, and small crystals retain their magnetization much better than do large ones. Where crust is forming above sea level, as it is in Iceland, molten rock often flows tens of kilometres before solidifying in thin horizontal sheets, which do not produce linear magnetic anomalies related to the field reversals. No one I know had expected that we would have the same luck on other planets, which is why the new observations^{1,2} are so unexpected.

What do the magnetic anomalies tell us about Mars? The planet must have had a core that acted as a dynamo early in its history, and so provides only the second unambiguous example of dynamo action inside a rocky planet¹. It must have had a liquid-iron core at this time. But the absence of a present-day magnetic field cannot be used to argue that the core has now solidified. We also cannot estimate the field's strength or the reversal frequency.

The magnetic anomalies themselves are surprisingly large (Fig. 1). Connerney *et al.*² remark that they require an intensity of magnetization that is an order of magnitude greater than that of the largest magnetic anomaly found on Earth — which is in Russia, and is ± 10 nanotesla at 100–200 km (the height of the Mars Global Surveyor). At that height, the magnitude of the martian anomalies is similar to that of anomalies produced by plate separation, but their width is much greater.

If the magnetization is similar, the anomalies require a magnetized layer that is 20–50 times thicker than that within the Earth's oceanic crust, which is generally believed to consist of a layer of quenched basalt about 2 km thick. On Mars, molten rock produced by plate separation will contain about twice the iron, but only half the titanium, concentration of terrestrial basalt⁴. Because of its smaller gravity field, plate separation on Mars will produce about 2.6 times the thickness of melt as it does on Earth if the mantle temperature is the same. So the martian crust is likely to be about 20 km thick, with twice the Earth's concentration of iron. Such a crust is adequate to generate the observed magnetic anomalies if it is magnetized as strongly as it is on Earth. Connerney *et al.*² suggest that iron sulphides are required as well, which is certainly possible. The shape of the martian anomalies suggests that the magnetization can resist remagnetization when the main field reverses. On Earth only the top couple of kilometres of the oceanic crust have the required magnetic stability, and it is hard to understand how the cooling on Mars can be more rapid than it is on Earth.

Another surprising feature of the martian anomalies is the lack of associated surface features. Lineated fault blocks parallel to the anomalies should be present if they are produced by plate separation, but there are no obvious topographic features of this kind. The anomaly profiles produced by Conner-

ney *et al.*² do not show obvious symmetry, as do those from terrestrial spreading ridges, but the coverage of Mars is still poor. Presumably it will remain so for some time, because the present spacecraft orbit is too far from the planet to be able to make accurate maps of the anomalies.

Am I convinced that the martian anomalies result from plate motions? Not yet. The shapes of the anomalies are not distinctive, they do not form obvious symmetric patterns and they are not clearly offset by fracture zones. A major problem with the

present data results from the variable height of the spacecraft: data are needed from a low circular orbit. Hopefully, the height will be reduced before the spacecraft fails! □

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Ageing

A message from the gonads

Donald L. Riddle

Animal evolution is commonly viewed as producing diverse, environmentally adapted bodies to propagate the germ line. The evolutionary theory of ageing suggests that genetic limits to lifespan may be inadvertent consequences of evolutionary selection for maximizing that propagation. In other words, trade-offs occur that favour reproductive success over post-reproductive longevity¹; lifespan should be inversely correlated with fecundity when progeny production diverts resources from the maintenance of somatic (non-reproductive) cells. The germ line contains all the genetic information to specify the soma. But it is also possible that there are other, environmentally modulated instructions for life history that the germ line conveys to the soma to maximize reproduction.

In their intriguing paper on page 362 of this issue², Hsin and Kenyon explore this possibility. From their data, it seems that the reproductive system of the nematode *Caenorhabditis elegans* regulates longevity by means of an insulin or insulin-like growth-factor (IGF-1) signalling pathway. Some general functional parallels between the nematode pathway and insulin signalling in vertebrates have been inferred from the phenotypes of the mutant nematode receptor³, but the functions of insulin-like sequences in the *C. elegans* genome have not yet been determined. Nevertheless, down-regulation of the pathway in *C. elegans* was known to result in long life^{3–5}, and now it appears that part of that signal is under the control of the gonads.

In *C. elegans*, sterile mutants do not live longer than their wild-type counterparts, implying that, under laboratory conditions, there is no trade-off between reproduction and longevity in this species. This was confirmed by Kenyon *et al.*⁴, who observed no effect on lifespan when they killed the four gonadal cells present at hatching — two germline precursors (cells that generate the sperm and eggs) plus two somatic-gonadal

precursors (cells that generate the reproductive organs) — with a laser microbeam. Hsin and Kenyon² have revisited this issue by destroying only the two germline cells, leaving the somatic gonad intact (see their Fig. 1 on page 363). Remarkably, this surgery extended life by about 60%, an observation they replicated in other wild isolates of *C. elegans* and in *Pristionchus pacificus*, a nematode thought to have diverged from *C. elegans* over 100 million years ago.

Removal of both somatic gonad and germ line has no effect, yet the intact germ line normally shortens life. So, what is happening? One explanation is that there is an equivalent signal from the somatic gonad to promote longevity in competition with the life-limiting germline signal. Killing the two somatic precursor cells alone is not a useful experiment, because the germ line will not proliferate in the absence of a somatic gonad. Instead, Hsin and Kenyon turned to a genetic argument using *daf* (dauer larva formation) mutants to address the question of a somatic-gonadal signal.

It has been known for some time that nematodes can regulate their lifespan. In an adverse environment, *C. elegans* can halt larval development to form the dauer (enduring) dispersal stage⁶. Dauer larvae do not feed, and can survive for months until food is found, whereupon they resume development. Post-dauer adults have the same two-week lifespan as adults that never entered the dauer stage, indicating that dauer larvae do not age⁷. Some of the *daf* genes encode components of the insulin/IGF-1 signalling pathway. Signalling inhibits dauer formation and also limits adult longevity. Insulin-like signalling may therefore regulate both development and ageing in response to nutritional status. This resembles the effect of caloric restriction on the lifespan of mice and rats, in which animals with restricted diets live longer than those fed as much as they want.

Mutants of *C. elegans* with reduced DAF-2 insulin-receptor activity live up to twice as

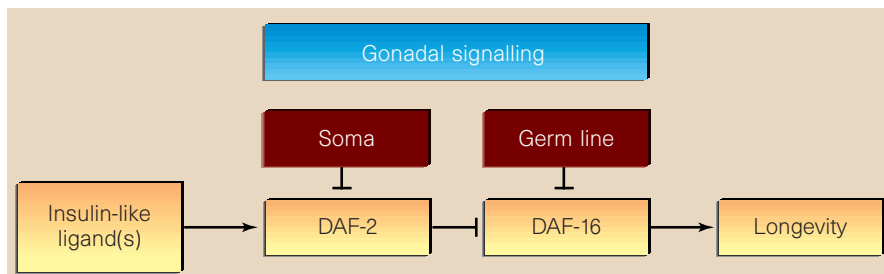


Figure 1 A model for gonadal influences on lifespan arising from the results of Hsin and Kenyon². Arrows, activating signals; T-bars, inhibitory signals. Insulin-like ligands are proposed to activate the DAF-2 receptor, which then deactivates the DAF-16 transcription factor through a phosphorylation cascade. DAF-16 increases longevity (by promoting a process that requires CTL-1 catalase activity) unless inhibited by DAF-2 or by a separate signal from the germ line. Somatic-gonadal cells enhance longevity by reducing the level of DAF-2 inhibition. The role of the DAF-12 protein is uncertain. Destruction of the whole gonad has a similar effect to *daf-12* mutations, so DAF-12 might be required for signalling from both somatic gonad and germ line. However, DAF-12 is not required for somatic-gonadal signalling in a wild-type genetic background. Hsin and Kenyon suggest that DAF-12 activity is influenced by DAF-2, an idea that is supported by other genetic data^{5,8}.

long as normal. They stay younger longer, as judged by their movement and appearance^{4,8}. The extended longevity requires the activity of DAF-16, a transcription factor in the forkhead family^{9,10}, and also involves DAF-12 (ref. 5), a nuclear hormone receptor⁶. The *daf-2* mutants overexpress CTL-1, a cytosolic catalase that may prevent oxidative damage, and CTL-1 activity is required for increased longevity¹¹. A *ctl-1* mutant has a 'progeric' (precocious ageing) phenotype. The overall conclusion in this context¹¹ is that the catalase protects the nematode from oxidative damage during dormancy, and increased protection extends adult life.

Hsin and Kenyon² have discovered that DAF-16 and DAF-12 activities are required for germline ablation to extend life. These proteins both appear to be transcription factors regulated by germline signalling. To reveal the hypothesized signals from the somatic gonad, they carried out a series of surgical experiments on animals lacking *daf* gene functions. Germline ablation did not extend the life of *daf-16* mutants, but whole-gonad ablation shortened life relative to that of intact *daf-16* controls, supporting the idea that the somatic gonad promotes longevity. Furthermore, ablation of either the germline precursors alone, or both the germline and somatic-gonadal precursors, extended *daf-2* mutant longevity, indicating that DAF-2 activity is required for the life-shortening response to destruction of the somatic gonad.

Given these results, the authors propose that signals from the normal somatic gonad promote longevity by inhibiting DAF-2 receptor activity. The idea is relatively simple. The germline signal shortens lifespan by downregulating DAF-16 and DAF-12 activities, whereas the somatic gonad produces a signal that lengthens life by inhibiting DAF-2 (Fig. 1). The activities of germ line and soma are opposite, because one acts on DAF-16,

which enhances longevity, and the other acts on DAF-2, which normally acts to shorten life.

One can imagine how gonadal signalling for longevity might be adaptive. Prolonged survival of the adult in response to limited food might improve its chances of reproducing. This is a strategy analogous to dauer arrest, and is apparently controlled by some of the same genes. On the other hand, a healthy germ line associated with maximal reproduction might also be adaptive by limiting a parent's competition with its progeny for food.

The dauer or non-dauer developmental decision, and the trade-offs between reproductive pattern and longevity, are major aspects of *C. elegans* life history. Investigations of how they have co-evolved, and the molecular controls that are responsible for their integration, are addressing one of the most complex issues in evolutionary biology. Perhaps it is not surprising that insights on ageing arise from developmental genetics. It is interesting to consider whether similar gonadal signals affect longevity in mammals, especially in species that hibernate. □ Donald L. Riddle is in the Molecular Biology Program and the Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211, USA.

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100 YEARS AGO

Thirty years ago, a French entomologist, named Leopold Trouvelot, was living at Medford, in Massachusetts. He was engaged in carrying on a series of experiments on rearing moths. ... He imported the Gipsy Moth, and by some accident, some of the insects escaped from his custody into his own or the neighbours' gardens. ... Had prompt measures been taken, the insect might possibly have been exterminated; but it does not seem to have attracted any attention till about 1880, when the people then living in or near M. Trouvelot's former residence began to be troubled with swarms of caterpillars, though what they were, and whence they came, was then unknown. For several years the neighbouring houses suffered severely, apple- and pear-trees and shade-trees being stripped of their leaves and killed, and the caterpillars creeping all over and into the houses. Nevertheless, they spread very slowly along the street, and into surrounding woods till 1889, when the insects multiplied so much that the caterpillars stripped all the trees in the immediate neighbourhood of M. Trouvelot's old house, and then marched forth in armies sufficient to blacken the streets, in search of fresh provender. A terrible account of the ravages of the caterpillars is given by those who witnessed them. From *Nature* 25 May 1899.

50 YEARS AGO

The numbers of university undergraduates, in the technical faculties especially, are to-day greater than ever before, and yet apprehension as to the true worth of university training was never so widespread as at the present time; for modern scholarship has made such strides, particularly in the field of science, that no one subject can be adequately studied without undue specialization and consequent neglect of other points of view. This danger of specialization was the theme of Mr. Oliver Stanley's recent address, on being installed as chancellor of the University of Liverpool, when he said that too many men and women to-day leave a university complete masters of a subject but still incomplete individuals, unable to act as evangelists of that broader culture, that more general philosophy which should be the university's gift to the people. From *Nature* 28 May 1949.

Condensed-matter physics

Taking the frustration out of ice

Mark Harris

Ferromagnetism — the ability of solids such as iron to be magnetized permanently — is the most familiar manifestation of magnetic order. It arises from a spontaneous alignment of the atomic magnetic dipoles (spins), driven by magnetic interactions between the atoms. But some magnetic solids display far more spectacular consequences of these interactions, particularly those that are geometrically frustrated. Put simply, this is the inability of magnets with certain crystal structures to attain magnetic order, solely by virtue of the geometrical arrangement of the magnetic atoms. The resulting behaviour is complex and rich in subtleties, pushing at the boundaries of our understanding of what constitutes crystalline order. It has implications far beyond magnetism — uniting many diverse problems, from the origin of freezing and glassiness to neural networks. Indeed, high-temperature superconductivity is believed by some to be due to a kind of frustrated magnetic state¹. But progress in the field has been slow, hampered by the experimental difficulties of isolating the frustration from other perturbations, such as defects, which are present in all real materials. On page 333 of this issue, Ramirez and co-workers² present convincing evidence that the pyrochlore compound $\text{Dy}_2\text{Ti}_2\text{O}_7$ corresponds to a new class of frustrated magnet called ‘spin ice’, which appears to be the cleanest laboratory yet for studying frustration.

Many magnetic materials in nature are antiferromagnetic, in which the spins find an ordering pattern where each is aligned antiparallel to its neighbours. Before the discovery of spin ice, only antiferromagnetic materials were considered eligible for frustration. To illustrate, consider how spins

behave when placed at the corners of some common structural building blocks: a square, a triangle and a tetrahedron (Fig. 1). With antiferromagnetic interactions it is possible for spins to align antiparallel on a square (Fig. 1a), but impossible on a triangle (Fig. 1b), because there is always one spin that cannot align antiparallel to both of its neighbours simultaneously. This inability to satisfy fully the interactions between spins — simply because of the geometry of the underlying structure — is the essence of geometrical frustration. But its effects become most severe in the pyrochlore lattice, which is composed of corner-linked tetrahedra. This can be seen from Fig. 1c, where we now find that two out of every four spins cannot align antiparallel. On the other hand, if we take ferromagnetic interactions between the spins in Fig. 1a–c, full order can be attained, because all spins can align parallel to each other. For this reason, ferromagnetism was always thought to be unfrustrated.

It was discovered that anisotropy (a further constraint on each spin’s alignment, and present to varying degrees in all magnetic materials) changes the accepted picture of frustration³, and in some cases turns it completely on its head. That is, even with the pyrochlore lattice, antiferromagnetic interactions can become unfrustrated and produce a conventionally ordered magnet, whereas ferromagnetic interactions can become strongly frustrated. This anisotropy is particularly strong in pyrochlores where the magnetic atom is a rare earth, notably Ho (holmium) and Dy (dysprosium). Here the anisotropy constrains each spin to point either directly into or away from the centre of each tetrahedron. With ferromagnetic inter-

actions, the four spins on each tetrahedron align so that there are two pointing in and two out. One of the six possible ways of achieving this on a tetrahedron is shown in Fig. 1d. When tetrahedra are linked to form a pyrochlore lattice, the large number of ways of satisfying the ‘two spins in, two spins out’ rule means that no single, ordered pattern is more favourable than any other. This is another definition of frustration. In fact, it can be shown⁴ that such a lattice is every bit as frustrated as any possible antiferromagnet, which came as a big surprise to those who had always blithely assumed that ferromagnetism had no surprises left.

More significantly, it became clear that this frustrated pyrochlore is a magnetic analogue of the old problem of how hydrogen atoms order in the ice (H_2O) lattice^{3,5} (hence the nickname ‘spin ice’). In this analogy, the spins represent hydrogen positions around a central oxygen atom, so ‘two in, two out’ corresponds to ‘two hydrogens close to, and two further away’ from each oxygen — the ‘ice rule’ that ensures the structure consists entirely of H_2O molecules. This rule does not favour one simple ordered structure, and, as Pauling realized in 1935, the number of possible structures is almost immeasurably large⁶. The entropy of ice (which is a measure of the molecular disorder of the system) was therefore expected to be finite at all temperatures down to 0 K, in agreement with the experiments of Giauque and co-workers⁷. The problem was that this finite entropy defies the third law of thermodynamics, which requires all substances to have precisely zero entropy at $T = 0$ K. Explicitly, zero entropy means there is only one equilibrium arrangement of the system of molecules.

For the past 60 years, this discrepancy has been reconciled by the proposal that the disordered arrangement does not reflect the true equilibrium state of ice at 0 K. The argument goes that if true thermodynamic behaviour could be attained, then more subtle effects would favour a unique ordered structure with truly zero entropy, and vindicate the third law. In light of the excellent agreement between Pauling’s finite entropy and the value found experimentally, this might seem churlish. But there is good experimental evidence that ice is indeed out of equilibrium at low temperatures. In particular, the hydrogen-ordering dynamics are prohibitively slow below about 120 K, suggesting that some sort of glassy non-equilibrium state is formed.

Spin ice may shed new light on this issue. The best experimental realizations of spin ice are the magnetic pyrochlore compounds $\text{Ho}_2\text{Ti}_2\text{O}_7$ and $\text{Dy}_2\text{Ti}_2\text{O}_7$. In 1969 it was noticed that $\text{Dy}_2\text{Ti}_2\text{O}_7$ has residual entropy at low temperatures⁸. Ramirez *et al.*² have uncovered a simple and beautiful result: this residual entropy is numerically in excellent agreement with the Pauling entropy for ice. So they provide the crucial link in the chain

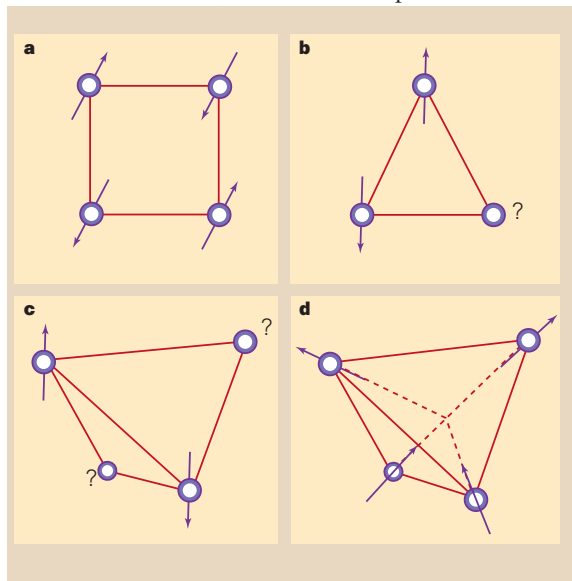


Figure 1 Three common crystal-structure building blocks (square, triangle and tetrahedron). The circles represent magnetic atoms and the arrows magnetic spins. In a–c the spins are coupled by antiferromagnetic interactions, favouring antiparallel alignment of neighbouring spins. This is easy in a, but impossible in b and c. In d the coupling is ferromagnetic, but there is the additional presence of anisotropy constraining the spins to point either directly into — or away from — the centre of the tetrahedron. The resulting configuration with ‘two spins in, two spins out’ is a characteristic of the ‘spin ice’ studied by Ramirez *et al.*².

between spin ice and water ice. This work reveals that two analogous systems — $\text{Dy}_2\text{Ti}_2\text{O}_7$ and ice — have the same entropy, even though the ordering dynamics of the spins and hydrogen atoms are vastly different. Yet these two materials still have the same statistical behaviour, pointing to a deeper truth behind Pauling's finite entropy. It is easy to believe that ice itself might be out of equilibrium, but it is harder to believe the same of $\text{Dy}_2\text{Ti}_2\text{O}_7$, because there is, as yet, no evidence of a transition into a glassy state like that of ice. One might conclude that spin ice presents a real threat to the third law of thermodynamics. That would be premature, but it certainly represents plausible evidence that the disordered state of water ice is the real ground state at low temperatures.

It would be no exaggeration to say that such a transparent and easily interpretable result has never before been obtained from a frustrated magnet. This highlights what is so appealing to the experimentalist about spin-ice compounds: despite the inevitable presence of defects and other minor perturba-

tions that frequently dominate other frustrated magnets, an inherent purity of behaviour comes through. The link between theory and experiment is therefore much more straightforward. It is clear that, because of this, spin ice has the potential to illuminate other vexing questions arising in the past 30 years. The most urgent is the origin of glassy behaviour in chemically ordered magnets, a question that reaches into the heart of what causes glassiness in the first place. □

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toxin genes⁷ — that is, they are expressed only when the bacterium finds itself in a suitable environment such as the epithelial surface of the intestine. The genes encoding the structural and assembly components of the TCP are located on a pathogenicity island known as the *V. cholerae* pathogenicity island (VPI)⁸. All strains of *V. cholerae* that can cause epidemic cholera contain a VPI, and it is thought to be transferred between strains in the environment. Certain features of the VPI and its chromosomal location suggest that this genetic element might be encoded by a bacteriophage^{1,8,9}.

Karaolis and colleagues¹ now show that the VPI is, indeed, a filamentous bacteriophage. Termed the VPI phage (VPIΦ), it can transmit itself between certain strains of *V. cholerae*, and it carries ssDNA in a similar way to CTXΦ. The protein that surrounds the DNA (the coat protein) is TcpA, a finding that has several astounding implications. First and foremost, it implies that the same structure may be both a colonization pilus and a bacteriophage particle. The authors propose that the bacteriophage can be formed and bud off from the bacterium while acting as a colonization pilus. If this is true, it indicates that the VPIΦ is the receptor for yet another phage — the CTXΦ (Fig. 1). But it is a mystery how an infectious phage particle, which must be released from the bacterial cell to infect another cell, can also act as a colonization factor and a phage receptor.

One possibility is that some of the TcpA produced by the bacterium serves one func-

Microbiology

Virus on virus infects bacterium

Ronald K. Taylor

Many bacteria carry segments of DNA called pathogenicity islands, which are enriched for genes encoding the proteins that contribute to virulence. Such proteins include secreted toxins, factors and signalling devices that help the bacterium to colonize its host, and even specialized types of molecular apparatus that inject bacterial products into host cells. We are starting to understand how the bacteria acquired these pathogenicity islands during evolution, and the latest findings are reported by Karaolis *et al.*¹ on page 375 of this issue. They describe a fascinating interplay between two pathogenicity islands — the first allowed the bacterium *Vibrio cholerae* to be infected with the second, which is a bacterial virus (bacteriophage) that carries the cholera-toxin genes.

Over 100 years ago, *V. cholerae* was identified as the bacterial species that causes the deadly, infectious diarrhoeal disease cholera². More recently, the potent cholera toxin was found to be encoded by a filamentous bacteriophage called cholera-toxin phage (CTXΦ), which exists within *V. cholerae*³. The CTXΦ contains single-stranded DNA, and it uses the bacterium's main colonization pilus — an appendage used to infect the host's intestine — as its receptor^{4,5}. This pilus, termed toxin co-regulated pilus (TCP), is a fibre of polymerized pilin protein termed TcpA. It promotes colonization by enhancing interactions between bacterial cells and, perhaps, directly with the host.

The TCP belongs to the type-4 family of pili, members of which are involved in colonizing diverse bacterial species⁶. As the name implies, expression of the genes that encode the TCP parallels expression of the

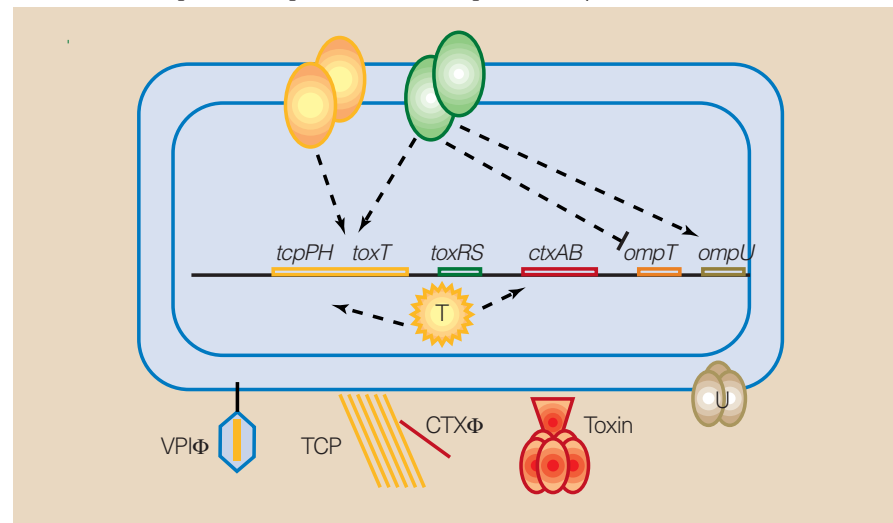


Figure 1 Regulation of virulence genes in pathogenic *Vibrio cholerae*. The *V. cholerae* pathogenicity island phage (VPIΦ) interacts with a recipient *V. cholerae* cell, resulting in infection and insertion of VPIΦ DNA into the bacterial chromosome. This allows production of the toxin co-regulated pilus (TCP), which, in turn, allows infection by the cholera-toxin phage (CTXΦ). The bacterium now has a full complement of virulence genes. Their expression is controlled by complex interactions between regulators encoded within the ancestral chromosome and those encoded on the newly acquired genetic elements. For example, the *toxR* gene product activates the expression of other genes encoding proteins in the bacterium's outer membrane (such as *ompU* in *V. cholerae*). In concert with the *tcpPH* genes (which are encoded by the VPI), *toxR* also activates expression of the VPI-encoded regulatory *toxT* gene. The ToxT protein (T) is required for expression of VPI genes, such as the *tcp* genes and the *ctx* operon of the CTXΦ, but it does not regulate expression of *ompU*^{7,11}.

tion, with the remaining TcpA serving the other. For example, not all strains of *V. cholerae* that express TCP can form transferable bacteriophage particles. Perhaps most of the TcpA is incorporated into a colonization pilus, and the remainder is used to coat the DNA in infectious phage particles (but only in those strains that can produce transferable phage). Interestingly, those strains that do not make accompanying phage were identified long before the strains that do. Maybe the bacteriophage has been caught in different stages of evolution — the newer strains can still produce the phage, whereas the more established strains have been selected for their ability to colonize host cells rather than for their capacity to transfer genes on a phage.

The VPIΦ is unusual in other ways. It is very large for a ssDNA filamentous phage, and the replicative form of its DNA is not very abundant. These properties may be related to the many functions of the genes that it encodes, or they may reflect a regulatory mechanism that balances development of the phage with formation of the pilus. Another amazing link between the VPIΦ and CTXΦ is the intimate co-regulation of gene expression. As shown in Fig. 1, virulence of *V. cholerae* involves a fascinating interplay between genes and regulatory elements encoded by the bacterial chromosome and those on the VPIΦ and CTXΦ. The fact that the TCP serves as the CTXΦ receptor indicates that, during evolution of toxin-producing strains such as *V. cholerae*, the VPIΦ has to be acquired before the CTXΦ can infect the bacterial strain. This sequence of infection ensures that the regulatory controls for interaction with CTXΦ genes are already in place.

The extent to which type-4 pili and bacteriophages are intertwined in other bacterial species remains to be seen. Many type-4 pili have other features, such as twitching motility, which may be involved in colonization or even in bacterial dispersal. But such functions would probably not be involved in phage production. In many cases, the genes that encode bacterial pili are not associated with a particular pathogenicity island. So, perhaps further links should first be pursued with those genes that are associated with pathogenicity islands. Whatever the outcome of such investigations, bacteriophages will continue to surprise us with their multifaceted roles in the evolution of pathogenic and other bacterial species¹⁰. □

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Palaeoclimatology

Warming without high CO₂?

Benjamin P. Flower

Over the next century, increasing levels of greenhouse gases are expected to raise Earth's mean surface temperatures by some 2–5 °C. To help understand future climate change, Earth scientists are examining ancient periods of extreme warmth such as the Miocene Climatic Optimum of about 14.5–17 million years ago (Myr). Fossil floral and faunal evidence indicate that this was the warmest time of the past 35 million years; mid-latitude temperatures were as much as 6 °C higher than at present. Many workers believe that high CO₂ levels, in combination with oceanographic changes, caused Miocene global warming by the greenhouse effect. But in the June issue of *Paleoceanography*, Pagani, Arthur and Freeman¹ present evidence for surprisingly low CO₂ levels of about 180–290 parts per million by volume (p.p.m.v.) throughout the early to late Miocene (9–25 Myr). These authors conclude that greenhouse warming by high CO₂ cannot explain Miocene warmth, and that other mechanisms must have had a greater influence.

In their study, Pagani *et al.* used a relatively new molecular-isotopic approach that has been effective in tracing CO₂ in glacial–interglacial cycles of the past several hundred thousand years^{2,3}. A record of surface water [CO_{2(aq)}] can be obtained from C_{37:2} alkenones in marine sediments, because isotopic fractionation during phytoplankton photosynthesis is largely controlled by [CO_{2(aq)}], cell growth rate and cell geometry. Assuming the effects of cell growth rate and cell geometry are minimal in low-nutrient waters, [CO_{2(aq)}] may be estimated by comparing C_{37:2} alkenone δ¹³C with the δ¹³C of total CO₂ as recorded by planktonic foraminifera. Atmospheric CO₂ can then be calculated from [CO_{2(aq)}] values based on Henry's law.

Pagani and colleagues' CO₂ record bears little relation to an expected association between CO₂ and long-term climate change during the Miocene (Fig. 1, overleaf). In particular, low CO₂ values span the warm Climatic Optimum which ended around 14.5 Myr, whereas rising CO₂ levels accompany global cooling and expansion of the East Antarctic Ice Sheet about 12.5–14 Myr (as inferred from the oxygen-isotope composition of benthic foraminifera from deep-sea sediment cores). Rising CO₂ during

the middle Miocene may eliminate a reverse greenhouse effect as the main cause of East Antarctic Ice Sheet expansion.

Several sources of uncertainty in calculating [CO_{2(aq)}] may increase CO₂ estimates, but only minimally. Miocene [CO_{2(aq)}] values may be artificially low if the δ¹³C of surface-water total CO₂ is overestimated. Foraminiferal δ¹³C may overestimate [CO_{2(aq)}] either because of species-dependent effects or because of the carbonate-ion effect on seawater δ¹³C (ref. 4). However, [CO_{2(aq)}] values are primarily controlled by variations in the δ¹³C of C_{37:2} alkenones, and even a 0.5–1‰ overestimation of surface-water δ¹³C would yield only slightly higher CO₂ values¹. Similarly, underestimation of sea-surface temperature for foraminiferal δ¹⁸O (due to a possibly lesser ice-volume effect) would increase calculated CO₂ only slightly.

Pagani and colleagues' low CO₂ values of 180–290 p.p.m.v. (present-day value is 360 p.p.m.v.) will fuel debate on long-term CO₂ history during the Cenozoic era. Some geochemical models^{5–8} suggest that increased global chemical weathering and erosion during the past 40 Myr progressively consumed atmospheric CO₂. Others argue that there was little change in either global weathering rates or CO₂ (ref. 9). Low Miocene CO₂ levels are consistent with palaeo-pH data¹⁰, which indicate little difference from the present-day ocean. Similarly, CO₂ trends on the timescale of 10⁵–10⁶ years are in line with foraminiferal δ¹³C records of organic-carbon burial relative to calcium carbonate (Fig. 1). High δ¹³C values between 13 and 17.5 Myr, termed the Monterey Excursion, indicate greater burial of organic carbon which may have been sufficient to draw down CO₂ from the atmosphere during this time¹¹. Thus, low Miocene CO₂ is supported by some independent geochemical data.

Furthermore, Pagani *et al.* find that short-term CO₂ minima coincide with glacial cooling on the 10⁴–10⁵-year scale. Coeval maxima in foraminiferal δ¹³C support the idea that organic-carbon burial was linked to CO₂ drawdown on such timescales^{12,13}. This finding squares with known mechanisms of climatic change during the past several hundred thousand years, in which high CO₂ clearly increased global warming. In short, CO₂ variability seems to contribute to

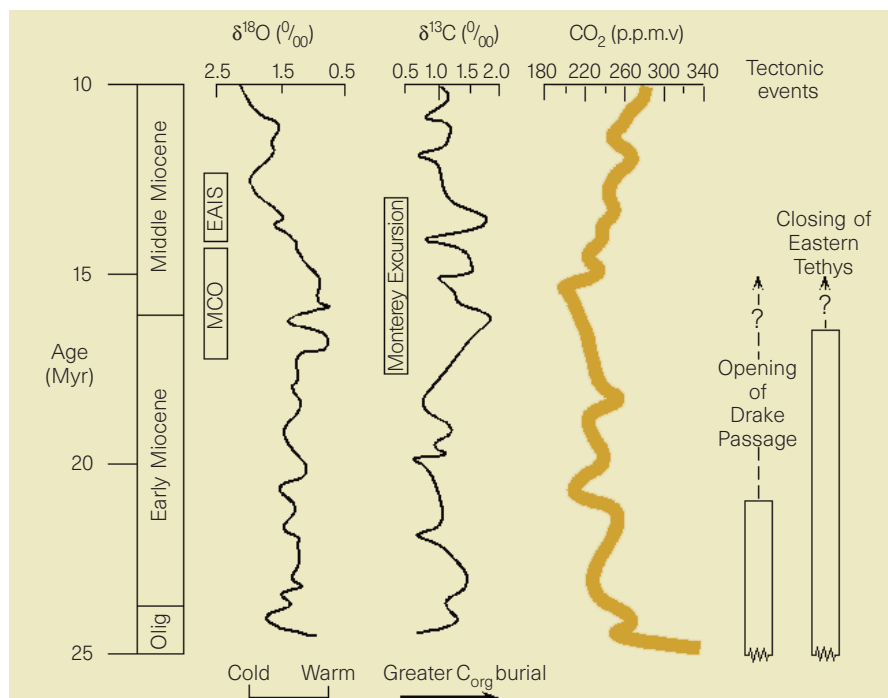


Figure 1 Tracing climate change in the Miocene. Shown here are records of ice volume and temperature (based on foraminiferal $\delta^{18}\text{O}$) and relative organic-carbon burial (based on foraminiferal $\delta^{13}\text{C}$), compared with the CO_2 estimates of Pagani *et al.*¹, and tectonic events that may have affected ocean heat transport. Trends in CO_2 are consistent with organic-carbon burial and CO_2 drawdown during the Monterey Excursion, but cannot explain the Miocene Climatic Optimum (MCO) or expansion of the East Antarctic Ice Sheet (EAIS).

climatic changes on the glacial–interglacial timescale, but may be partly decoupled from long-term climate change.

If Pagani *et al.* are right, then how can one explain the high temperatures of the Miocene Climatic Optimum, or middle Miocene global cooling and expansion of the East Antarctic Ice Sheet? Other possible controls include different greenhouse gases (water vapour and methane, for example), and ocean circulation. On million-year timescales, the openings and closings of ocean ‘gateways’ caused by changes in continental geography may affect ocean heat transport. There are large uncertainties in dating these events, but the opening of the Drake Passage (between 22 and 32 Myr; ref. 14) and closure of the eastern Tethys Ocean probably reduced meridional ocean heat transport to the Southern Hemisphere^{15–17}, and may account for a large part of Miocene climate change. As Pagani *et al.* point out, low CO_2 levels may also have increased the sensitivity of the climate system to heat and vapour transport. Alternatively, changes in atmospheric water vapour and moisture supply to the Antarctic continent may have promoted both global cooling and growth of the East Antarctic Ice Sheet.

The exciting possibility that CO_2 levels were low during the Miocene has implications for our understanding of many elements of Earth system history, including climate, ocean chemistry, global chemical weathering and the evolution of CO_2 -sensi-

tive biota. It also raises the prospect that projected CO_2 levels in the next century (perhaps 560–720 p.p.m.v.) may be the highest of at least the past 25 Myr. Associated greenhouse warming may therefore reach the levels of the early Cenozoic. Nevertheless, low Miocene CO_2 underscores the potential importance of different greenhouse gases, ocean circulation and other mechanisms in past as well as present global change. □

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Daedalus

Untrue feelings

The nerve-traffic of the body is very hard to interrogate or subvert. A nerve trunk may contain hundreds of fibres; an electric shock can fire them all together, but cannot address them individually.

Daedalus now has a scheme to do it. Each fibre, he points out, has its own speed of pulse transmission. Imagine, he says, a sequence of electrodes placed along the subject’s arm, for example, above the median nerve. Apply a small voltage spike to each electrode in succession, so that the stimulation travels up the arm at a specific velocity. If the individual pulses are too weak to fire any single fibre, the subject will feel no shock. But suppose the voltage spike steps along at the exact transmission speed of one specific fibre. A matching disturbance will be launched up that fibre, and will be amplified by each successive electrode in travelling-wave-tube manner, until it becomes a fully self-sustaining nervous impulse. Other fibres with different impulse velocities will not keep pace with the moving stimulation, and will not be fired. The subject will feel the sensation carried by that fibre alone — a tickle in his thumb, say.

This elegant technology opens the way for true ‘virtual sensations’ superimposed on the normal sensory traffic of the nervous system. Once the velocities of the fibres in a nerve trunk have been mapped, a computer-controlled electrode array above that nerve could fire just the fibres needed to generate any desired sensory illusion. The long run of the spinal cord, which carries so much nervous traffic, is ideal for travelling-wave-tube stimulation. Aldous Huxley’s ‘feelies’ — cinema with sensations — would become possible, computers could communicate with their users by tactile signals, and real telephone sex would be feasible at last.

Virtual sensation, however, will be most useful in tactile education. Doctors, mechanics, cooks, violinists and so on, all have to learn special sensory skills. A set of travelling-wave nerve programs, recorded from experts in the field, could teach the novice the actual feel of a fast pulse, a slack bearing, a too-sticky cake mix or a delicately sustained bow stroke. But sight and hearing, the most important senses, are badly suited to the technique. Their nerves run too deep in the skull to be reached by surface electrode arrays. Nerve-induced virtual television and audio, by-passing the eyes and ears and piped in 24 hours a day, are (perhaps mercifully) unfeasible.

David Jones

Topology in chaotic scattering

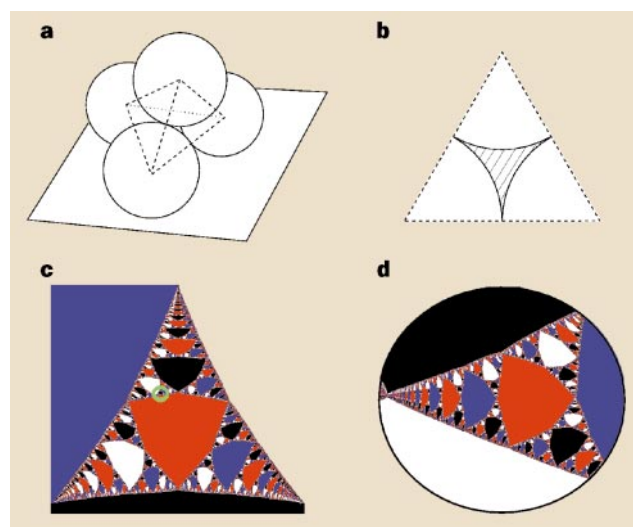
In chaotic scattering, an initially freely moving orbit (such as that of an atom or a star) enters a scattering region and evolves chaotically for a period of time before it escapes and returns to free motion¹. We have looked at cases in which escape can occur in three or more distinct ways. Using a laboratory model, we demonstrate experimentally that the regions of state space (called basins) corresponding to different ways of escaping can have an interesting topological property that we call the Wada property, by which we mean that these regions of state space might be so convoluted that every point on the boundary of a basin is on the boundary of all basins^{2,3}.

Chaotic scattering enables us to model complex phenomena in several physical systems, including chemical reactions⁴, celestial mechanics⁵, fluid dynamics⁶ and electron scattering in semiconductor microstructures⁷. In our laboratory model, we consider the path of a light ray as it reflects from a scatterer consisting of four mirrored balls⁸ arranged as in Fig. 1a. A light ray entering the 'inner chamber' between the four balls can leave through one of four openings (Fig. 1a, b). Thus we can identify four basins, each consisting of the collection of light rays that leave through one of the four openings. The boundary separating these basins has the Wada property, which is important in chaotic scattering situations because it impedes one's ability to predict the escape mode (for our model, the opening through which a light ray will leave the scatterer) from an initial condition.

It has previously been shown (reviewed in refs 3 and 9) that a division of space into three or more regions can have this strange topology. A theorem has been proved (reviewed in ref. 10) that implies that the boundary separating the basins (in the complex plane) of the four roots, found by using Newton's method for a fourth-degree polynomial, has the topology of the basin boundary that we examine here.

The photograph in Fig. 2 shows a view looking in through one of the openings of the scatterer. Two of the colours, red and blue, are the reflections of poster boards placed outside two of the openings. Light reflects from a poster board; then, having taken the board's colour, it reflects off the spheres, perhaps many times, before entering the camera lens. (Similar effects create the white patches: we passed light through a white diffusive filter before allowing it to pass into the scatterer by the third opening.) The black areas result from our having left the fourth (viewing) opening uncovered and the room dark. Had light been shone

Figure 1 The light-scattering system used in our laboratory model. Four mirrored spheres (approximately 15 cm in radius) are stacked like cannonballs, three resting on the table and the fourth placed on top of the three so that they touch each other. **a**, A diagram of the scatterer. The dashed and dotted lines show how the sphere centres are located at the vertices of a tetrahedron. A light ray can enter or leave the inner chamber of the scatterer through one of four openings lying in the four faces of the tetrahedron.



Once a light ray leaves, it cannot return to the inner chamber. **b**, Diagram of the shape (shaded) of one of the openings on a tetrahedron face. **c**, Plot of basins created by using numerical simulation. Light rays are initiated on a portion of the surface of one of the spheres that faces into the scatterer. Each light ray is aimed away from the viewer. **d**, If a small-diameter laser beam were shone on the circled region in **c**, parts of the beam would land in each of the four basins, as indicated by the presence of all four colours in this enlargement of the circled region. Enlargements of successively smaller sections of the boundary always contain all four colours. This suggests that the basins have the Wada property, where every point on the boundary of a basin is on the boundary of all basins.

on the camera and photographer, their reflections would have been visible in the black areas.

In Fig. 2, the four colours create a map of the four basins. If an observer were to aim a narrow beam of light from his or her eye to a blue patch, for instance, that beam would hit the blue poster board after some reflections. It would leave the scatterer through the 'blue' opening and be said to have started in the blue basin. The boundary between the four colours is a fractal, and its fractal dimension, determined from numerical simulation, is approximately $D=1.6$. Thus, our work provides a simple laboratory demonstration of an interesting type of fractal geometry.

To study this system numerically, we used the geometrical optics approximation (in other words, diffraction is neglected) in which the trajectory of a light ray reflecting from the spheres corresponds to that of a point particle bouncing off the spheres. We plotted a picture similar to that in Fig. 2 (see Fig. 1c) and magnified regions containing points in the basin boundary (for example, Fig. 1d). We find that no matter where, or by how much, we magnify around a boundary point, we always see all four basin colours. Thus, arbitrarily close to any boundary point we find points in all four basins, so every boundary point is on the boundary of all four basins.

Imagine performing an experiment on a scattering system with the Wada property.

We would measure the initial conditions within some error bounds and record the outcome of the experiment. If the initial condition were sufficiently near the basin boundary then the small random error in the initial condition could push the experiment

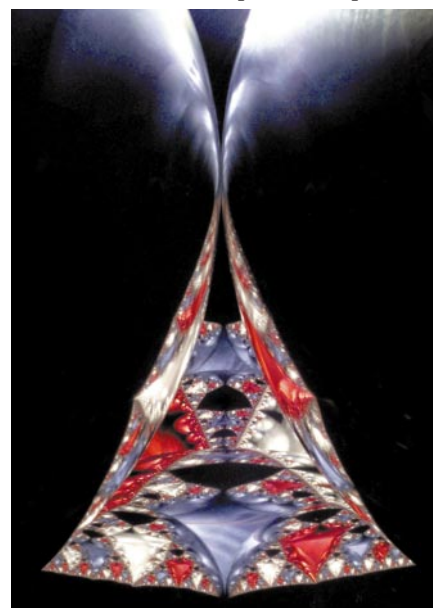


Figure 2 Photograph of the laboratory model of the scatterer described in Fig. 1. Red and blue poster boards were placed outside two of the upper openings and a white cloth was placed below. Their reflections created the coloured pattern seen through the fourth opening. The boundary separating the colours has a fractal dimension of ~ 1.6 .

into any of the possible outcomes because of the Wada property.

We have verified the Wada property experimentally for our laboratory model by using a beam of 0.48 mm diameter from a 0.95 mW helium–neon laser. If the beam is shone on a boundary point, we find that the laser light can be seen through each of the openings. However, if the beam is aimed at the interior of one of the coloured regions, the light is seen through only one opening, casting a bright spot on the corresponding poster board.

We believe that Wada boundaries should be a typical feature in chaotic scattering systems that have more than two exit modes. We have verified the existence of Wada boundaries in a situation involving chemical reactions⁴ with two and three degrees of freedom.

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Rainfall characteristics of hurricane Mitch

The hurricane or tropical storm known as Mitch struck Central America towards the end of October 1998. The subsequent flooding and landslides claimed approximately 11,000 lives. It was the most deadly hurricane to strike the Western Hemisphere in two centuries. We have measured rainfall totals during Mitch (from 1 to 48 hours) and find that they were not exceptional for hurricanes and tropical storms in the Atlantic basin. Rainfall totals and intensities measured over intervals of 1, 2, 5, 10, 30 and 60 minutes were less than values from the updated maximum potential rainfall curve^{1,2}. The data suggest that extraneous factors, such as already saturated soils and denuded hillsides, were largely responsible for the damage caused.

Detailed rainfall data from hurricanes are often lacking because rainfall-recording instruments are washed away. The emphasis has been on recording wind strengths, which can underestimate the damage potential.

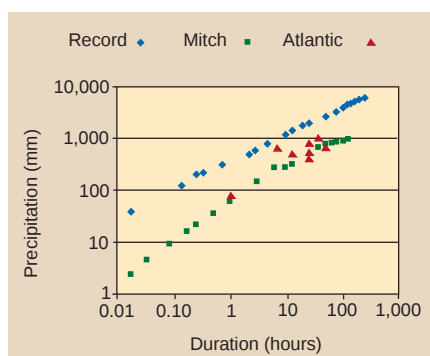


Figure 1 Plot of maximum rainfall amounts (squares) against duration from a rain gauge in southern Honduras during hurricane/tropical storm Mitch ($y = 51.64x^{0.054}$, $R^2 = 0.99$). Also plotted are updated record rainfall events (diamonds) for different durations¹² that define the curve of maximum potential rainfall ($y = 353.07x^{0.059}$, $R^2 = 0.99$) and data (triangles) from recent major Atlantic hurricanes and tropical storms^{3–5}.

Rainfall distribution is often estimated from radar and satellite data with little ground-truth support. Data from an autographic rain gauge in southern Honduras, where flooding and landslides were extensive, provide a unique insight into the rainfall distribution during Mitch. The tipping-bucket rain gauge (ELE DRG-52) was located at 87° 04' W and 13° 17' N on a hill crest (100 m above sea level) in the foothills of Cerro Guanacaure (1,007 m above sea level).

Mitch formed on 21 October 1998 in the southwest Caribbean and reached category 5 hurricane status on the Saffir/Simpson scale as it moved towards northern Honduras. On 29 October, Mitch was downgraded to a tropical storm and moved southwards and inland. The storm was declared to be over on 1 November.

Mitch produced torrential rains despite being downgraded from a hurricane. Between 18:00 (Honduran time, which is 6 hours before GMT) on 27 October and 21:00 on 31 October, there was 896 mm of rainfall. The most intense and prolonged rainfall was between 15:00 on 29 October and 07:00 on 31 October (a total of 41 hours). During this period, 698 mm of rain fell. There were two distinct periods of extreme rainfall intensities: 186 and 245 mm of rain fell during six-hour periods from 16:00 to 22:00 on 29 and 30 October, respectively. Maximum intensities ranged from 138 mm h⁻¹ (2-minute period) to 58.4 mm h⁻¹ (60-minute period). Landslides affected approximately 20% of surrounding hillsides and typically occurred during the two periods of most intense rainfall. Simultaneously, the Choluteca river flooded adjacent settlements and parts of the city of Choluteca. The river also altered its course.

Although Mitch caused extensive flooding and loss of life, Fig. 1 shows that rainfall

in southern Honduras was comparable to the severest hurricanes and tropical storms in the Atlantic basin^{3–5}. Rainfall was also substantially less than the updated maximum potential rainfall curve, which is defined largely by rainfall events from La Réunion, Indian Ocean^{1,2}, where there might be greater topographic forcing. The data suggest that extensive damage in Honduras and Nicaragua was accentuated by several factors: the storm struck at the end of the rainy season when the soil was saturated, resulting in catastrophic flooding and landslides; agricultural extension caused by land pressures had left many hillsides denuded; and the population was ill prepared because, before making landfall, it had been predicted that Mitch would move northwards.

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Analysis of telomere lengths in cloned sheep

The development of nuclear-transfer techniques using cultured somatic cells^{1–4} allows animals to be produced without involving germline cells. This enables us to examine the importance of the repair of chromosome ends (telomeres) in the germ line and to test the telomere hypothesis of ageing^{5–8}.

To investigate whether telomere erosion is repaired after nuclear transfer, the telomere lengths of animals 6LL3 (Dolly)¹, 6LL6 and 6LL7 were compared with age-matched control sheep, donor mammary gland tissue and donor cells before and after culturing (Fig. 1a). Animal 6LL3 was derived from the transfer of a nucleus from sheep (ovine) mammary epithelial (OME) cells from a six-year-old Finn Dorset sheep; 6LL6 was derived from sheep embryonic cells (SEC 1) obtained from day 9 Poll Dorset embryos; and 6LL7 was derived from fibroblasts¹ from a day 25 Black Welsh fetus.

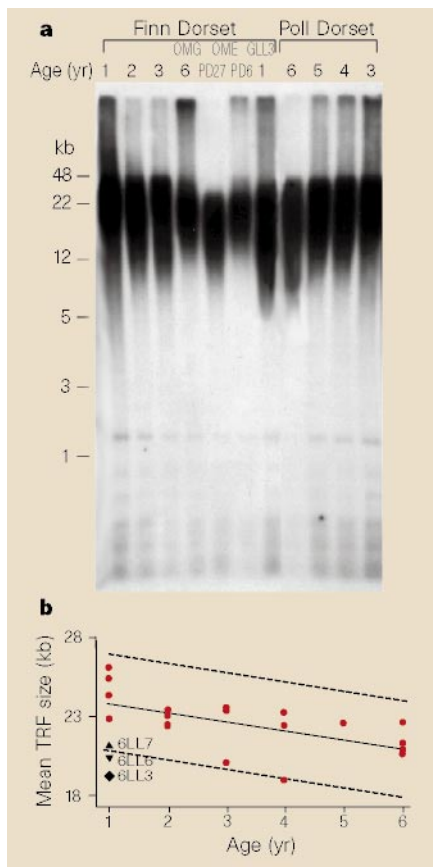


Figure 1 A representative analysis of sheep TRF lengths. **a**, Genomic DNA from Finn Dorset sheep, the six-year-old Finn Dorset ovine mammary gland (OMG) tissue used to provide donor cells for nuclear transfer, OME cell primary cultures derived from the aforementioned tissue, Finn Dorset nuclear-transfer animal 6LL3 (Dolly), and from Poll Dorset animals was analysed by Southern blotting and hybridization with radiolabelled (TTAGGG)₃ oligonucleotide. Animal ages are indicated above their respective lanes; the duration in culture for primary cells (OME) is indicated as population doublings (PD). The presence of a consistent signal at approximately 1.5 kb was used as a comparative control for loading and sample integrity. **b**, Regression analysis of mean TRF lengths against age, showing the decline in telomere length with age for the controls (solid circles) together with the fitted line (solid line) and 95% prediction interval for an additional observation at any given age (dashed lines). Comparisons in the text between nuclear-transfer sheep and controls were made by using the prediction intervals from the regression and by Student *t*-tests on 17 degrees of freedom; the control sheep were used to estimate the mean response and its variance. Similar conclusions for 6LL3 were drawn by using a two-sided *t*-test against either the four controls aged 1 year or the four controls aged 6 years, with the use of the appropriate group of four control sheep to estimate the mean response and its variance.

The mean size of the terminal telomere fragment obtained by cutting with restriction enzyme (the mean terminal restriction fragment, or TRF) was found to decrease in control animals with increasing age, at a mean rate of 0.59 kilobases (kb) per year. A linear regression analysis of the sheep DNAs yielded a significant result ($t=3.29$; $P<0.01$) (Fig. 1b).

Mean TRF sizes were smaller in all three nuclear-transfer animals than in age-matched controls. DNA in 6LL3 showed the greatest diminution of mean TRF size for a one-year-old animal (19.14 versus 23.9 ± 0.18 kb). The size difference is significant ($P<0.005$) compared with the age-matched control animals. The smaller TRF in 6LL3 is consistent with the age of her progenitor mammary tissue (six years old) and with the time that OME cells derived from that tissue spent in culture before nuclear transfer. 6LL6 also showed a significant decrease in TRF size (20.37 versus 23.9 ± 0.18 kb; $P<0.030$). As the number of animals analysed was small, it is possible that the difference was due to natural variation of the mean TRF size in these individuals, but the statistical significance of the data argues against this.

There was no significant difference between the DNA from the six-year-old progenitor mammary tissue and age-matched control DNA from fresh blood.

The influence of duration in culture, superimposed on the effect of the age of the

progenitor tissue, can be gauged from the diminution in mean TRF size of OME cells that have undergone up to 27 population doublings. A decrease in mean TRF size was observed at an average 0.157 kb per population doubling. This is an average derived from replicate experiments and is consistent with results from human somatic cells in culture^{9,10}.

These observations indicate that the extent of shortening of the TRF might be mitigated, principally by minimizing the duration in culture and by a careful choice of the source of donor cells. This is particularly relevant for 6LL7, for which the use of fetal tissue and minimal culturing yielded an animal in which the mean TRF size was not significantly shorter at 95% confidence limits (21.19 versus 23.9 ± 0.18 kb; $P<0.088$) than age-matched controls. This is in contrast to results from 6LL3 and 6LL6, for which culturing was more prolonged.

The most likely explanation for the shorter mean TRFs in all three nuclear-transfer animals is that the mean TRF size observed in these animals reflects that of the transferred nucleus. Full restoration of telomere length did not occur because these animals were produced without germline involvement. It remains to be determined whether any telomerase activity, or an alternative telomere-lengthening mechanism, is present that could result in some telomere repair during the early development of sheep.

It is not known whether the actual physiological age of animals derived by nuclear transfer is accurately reflected by TRF measurement. Recent veterinary examination of the nuclear-transfer animals has confirmed that they are healthy and typical for sheep of their breeds, despite having a shorter mean TRF length. Furthermore, 6LL3 has undergone two normal pregnancies and has successfully delivered healthy lambs.

Telomere-based models of cellular senescence⁵⁻⁹ predict that the nuclear-transfer-derived animal 6LL3 would reach a critical telomere length sooner than age-matched controls. However, considering the large size distribution of sheep TRFs, it remains to be seen whether a critical length will be reached during the animal's lifetime. The experimental inactivation of murine telomerase produced a phenotype only after five generations¹⁰, and similar observations have been made in telomerase-deficient yeast cells¹¹. Mice have also been sequentially cloned by the transfer of adult cumulus-cell nuclei without any adverse effects¹².

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Did parrots exist in the Cretaceous period?

The timing of the origin of modern birds is much debated. The traditional view, based largely on the fossil record, suggests that most modern groups did not appear until the Tertiary, after the end-Cretaceous extinction event¹, but recent work, based on molecular divergence data, has suggested that most, or all, of the major clades were present in the Cretaceous^{2,3}. Verification of the latter proposal awaits the discovery of modern bird fossils in the Mesozoic which can be confirmed on the

basis of the derived features characterizing the major clades.

We do not consider that the recent description⁴ of an avian dentary symphysis of a supposed psittaciform (parrot-like) bird from the Cretaceous Lance Formation of North America represents such a record. If it did, then this would be not only the oldest record of parrots by some 15 million years, but also the earliest recorded occurrence of a 'terrestrial' modern bird in the Cretaceous. Other reports from this period have been shown to be too fragmentary to be of any taxonomic value^{5,6}, to occur within formations of uncertain Cretaceous age^{7,8}, or to have been incorrectly assigned in the first place⁹.

We therefore recommend that this record be treated with caution until further fossil material of a similar age can be assigned with confidence to the Psittaciformes. This record should not be used to support hypotheses invoking an origin for the modern clades before the Cretaceous/Tertiary boundary^{2,3} for the following reasons.

First, the characters listed by Stidham⁴ to support the referral of this material to the Psittaciformes have a wider distribution among Cretaceous Maniraptoriformes (for example, a 'hook-like' dentary is seen in caenagnathid theropods¹⁰), and are variable within the group in question. Although a K-shaped neurovascular canal pattern is seen in some modern psittacids (such as *Cyanoramphus* sp.), this character is not seen in other taxa (*Polytelis* sp., for example). In a survey of large numbers of skeletal specimens of several modern species, we have observed that a K-shaped neurovascular canal pattern is variable in occurrence within individual psittaciform taxa (for instance, *Psittacula roseata*; G.J.D., personal observation), and that both the putative psittaciform characters cited by Stidham⁴ are seen in other groups of modern birds (such as the Ciconiiformes; G.J.D., personal observation).

Second, the overall morphology of the Lance Formation specimen is markedly different from that of the oldest unequivocal parrots known in the fossil record, including well-preserved and complete skulls from the lower-middle Eocene of the London Clay, England, and Grube Messel, Germany¹¹. These fossil birds, although exhibiting a typically psittaciform postcranial morphology (including the zygodactyl foot, which has the fourth toe directed backwards), lack the parrot-like beak of modern Psittaciformes. Moreover, the mandibular symphysis in Eocene forms is much smaller and narrower than in recent parrots and in the specimen from the Lance Formation^{4,11}.

At present, the monophyly of the Psittaciformes, one of the most homogeneous of modern orders, is supported

exclusively by postcranial characters¹¹, although the single recent family within the order, the Psittacidae, does have a single unique skull character: the presence of a 'parrot-like' beak (for example, maxilla broad dorsoventrally, with a sigmoidally curved ventral margin). We argue that, given the benefit of well-preserved and largely complete fossil material from the early Eocene, taxonomic assignments of material such as the Lance Formation specimen must remain tentative at present.

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Stidham replies — I have presented a hypothesis for the identification of a Late Cretaceous fossil as the oldest known parrot¹. The specimen lacks the characters distributed more widely in non-avian maniraptoriforms, such as abundant teeth and unfused dentaries. It has many characters¹, including the absence of an internal pillar of bone supporting a midline ridge, that are not present in oviraptoroids². To assign the specimen to a non-avian clade requires a less parsimonious hypothesis of character evolution. The K-shaped neurovascular canal pattern character¹, mapped onto various phylogenetic hypotheses of the relationships of crown group parrots^{3–5}, is primitive for that clade. Like most characters, the K-shaped neurovascular canal pattern exhibits homoplasy and variation within natural populations. The complete absence of this character from some extant parrots seems to be the result of secondary loss due to the relative shortening of the jaw symphysis in some parrots (*Neophema*, for example). However, this character, as figured and described¹, is not found outside crown-group parrots, although it superficially resembles the state in extant cathartid vultures. Although individual characters seen in the fossil can occur in other taxa, the combination of characters seen in the fossil

is not present outside crown-group parrots, and Dyke and Mayr have not demonstrated what clade, other than parrots, has this combination of characters.

It seems less than defensible to propose that we cannot have Cretaceous parrots because the oldest well-preserved fossils known so far are Eocene⁶. Previously proposed sister groups to parrots (reviewed in ref. 7), and the known fossil record of these sister taxa^{8,9}, show that the parrot lineage should have been present at least 5 to 10 million years before the (middle Eocene) Messel and London Clay parrots⁶. This is the same amount of missing fossil record required by both my hypothesis of a latest Cretaceous parrot and Mayr and Daniels' suggestion⁶ (made during their study of the Eocene parrots) of a Cretaceous origin of parrots.

The other known Cretaceous neornithines (listed earlier¹: in contradiction with Dyke and Mayr, the New Jersey fossil birds are Cretaceous in age¹⁰) placed in various phylogenetic hypotheses of the ordinal level relationships of neognaths, including parrots^{7,11}, show that parrots and most other neognath ordinal level clades are constrained to have diverged from other orders of modern birds in the Cretaceous or early Palaeocene.

If non-crown-group parrots are present in the Eocene⁶, then the sister group to those taxa (the stem leading to the crown group or the crown group itself, possibly with a modern-looking jaw) must have been present by the middle Eocene as well. The identification of the Cretaceous jaw as a parrot is subject to test and refutation, like any hypothesis. However, the accepted methods of the field, not statements about gaps in our current knowledge and preconceived notions of character evolution, must be used to falsify hypotheses and generate alternatives.

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Grain of truth in fears of biotechnology

Against the Grain: The Genetic Transformation of Global Agriculture

by Marc Lappé and Britt Bailey
Earthscan: 1999. 160 pp. £15.99

John Mugabe

Few twentieth-century technologies have attracted as much controversy as biotechnology. International debate has two extremes: one group regards biotechnology as the source of solutions to the world's problems of environmental degradation, disease and food insecurity. The other sees it as a threat to global and local economic and environmental security. Those in the latter group cite a catalogue of dangers: displacement of traditional export crops from developing economies, the ecological and health risks associated with release of genetically engineered organisms, and the privatization of knowledge by corporations.

Against the Grain adds to this debate, and specifically to discussions of the negative consequences of genetic engineering in agriculture. Marc Lappé and Britt Bailey adequately cover the ethical problems and the potential health and ecological threats that genetic engineering poses to the future of US and world agriculture, and examine US public awareness of and response to its impacts. They suggest that Americans have not paid sufficient attention to the problems of genetic engineering, owing to factors such as absence of media coverage, a young food culture and limited historical experience with technological hazards.

In documenting the scientific context and corporate motives for genetic engineering of crops, Britt and Lappé question the short-term-profit motives that drive corporations such as Monsanto and DuPont to select certain genes and crops for genetic engineering. While I agree that these companies should be guided by ethical and environmental considerations, it is important to recognize that — in the absence of informed public concern to challenge corporate decisions — private companies are hardly likely to set high standards for themselves.

Biotechnology companies all over the world are engaged in intense competition, because the technology is advancing rapidly and uncertainly in a dynamic global market. Their investments in genetic engineering are enormous, and they have to reap profits if they are to survive. It is essentially to maintain their competitive edge that companies such as Monsanto are exploiting the benefits of technological convergence to concentrate on genetic engineering of crops and genes that confer



Test-tube plants: have the potential risks of genetically engineered plants been examined carefully enough?

resistance to their own herbicides.

This strategy is not unique to biotechnology companies. Those in many other sectors exploit the advantages of prior scientific knowledge and market concentration to develop a wide range of products. It is cheaper for them, as they often invest in incremental innovations instead of charting new technological trajectories. Biotechnology companies — particularly those engaged in genetic engineering, such as Monsanto — have therefore to commit their resources to rapid commercialization of a particular product. It is the challenge of the public and governments to create incentives for them to diversify into new crops and herbicides.

Against the Grain is silent on the role of public research — and particularly university laboratories — as major sources of new scientific knowledge and information for corporate activities in agriculture and other sectors. While many big companies and some of the dedicated biotechnology companies have, over the past 10 years or so, accumulated in-house research capability in life sciences, they continue to draw on universities for new scientific leads. They do this through specific agreements with university-based scientists and sometimes through collaborative agreements with whole research faculties. Therefore, any efforts at regulating genetic engineering of crops and foodstuffs should target public research as well. If ethics are built into the science they can easily get embodied in the resultant technology.

The book identifies the lack of a coherent

institutional framework for the US administration to enforce existing regulatory measures. Regulation of genetic engineering in agriculture falls within the purview of at least three agencies: the Environmental Protection Agency (EPA), the Food and Drug Administration and the US Department of Agriculture (USDA). The authors' careful analysis of cases of lack of regulation shows that agencies are lacking in technology-assessment capability. Agencies may not be abdicating their responsibilities to regulate corporate behaviour — nor compromising on enforcement of the rules, as the authors may want the reader to believe. Rather, they do not have the capacity to conduct assessment of the ecological, health and consumer impacts of genetically engineered crops.

Companies such as Monsanto and DuPont do possess considerable scientific expertise for assessment. But in the absence of standards and independent risk assessment, one should not expect them to use this for the public good through emerging profit-making opportunities. The challenge for the US administration is to create independent technology-assessment capability to enable such agencies as the EPA and USDA to control the development and release of genetically engineered foodstuffs.

Against the Grain ignores both the positive prospects that genetic engineering offers to agriculture and the way benefits from the technology can be maximized. The ethical and environmental ills of genetic engineering of crops are overemphasized. The authors acknowledge that there is scientific



uncertainty about the advances made in this area, but they still provide a catalogue of ills as if there were established scientific and empirical evidence about the impacts of genetic engineering. A precautionary approach would better inform policy and public debates on genetic engineering.

The challenge lies in establishing regulatory systems, including codes of conduct, to guide scientific exploration and corporate investment in biotechnology. Indeed, informed administrative and legislative supervision are urgently required to maximize benefits and reduce negative impacts from corporate genetic engineering. Moreover, as the authors say, "the coming genetic revolution can be an enlightened one only if the public understands its implications from its inception". The technology is here with us; it is not possible to stop its growth, even if that were desirable.

This book is timely because of the growing urgency to establish systematic measures to regulate global developments in and application of genetic engineering, as well as to ease tension between corporate economic interests and public ethical and environmental aspirations. Although this book tells a one-sided and sometimes alarmist story, it provides convincing evidence that governments around the world must, urgently, create and enforce regulatory measures to control irresponsible corporate investment in genetic engineering of food. □

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Ploughing the same field

Applied Plant Biotechnology

edited by V. L. Chopra, V. S. Malik & S. R. Bhat

Science Publishers, £58

Transgenic Animals in Agriculture

edited by J. D. Murray, G. B. Anderson, A. M. Oberbauer & M. M. McGloughlin
CABI, £55, \$100

Private Science: Biotechnology and the Rise of the Molecular Sciences

edited by Arnold Thackray
University of Pennsylvania Press, \$52.50

A call for unification

The Uncertain Sciences

by Bruce Mazlish

Yale University Press: 1999. 328 pp. \$35, £25

Nicholas Rescher

In *The Uncertain Sciences*, Bruce Mazlish construes the "human sciences" as including "all descriptive efforts to understand specialized aspects of human behavior — political, economic, sociological, and so on". This "and so on" covers a lot of ground here, since "even literature can at least be imagined as a legitimate form of science". On this basis, the book focuses on the questions: "What sort of knowledge do the human sciences claim to be offering? To what extent can that knowledge be called scientific?"

In seven brisk chapters, Mazlish develops his principal thesis that the human sciences occupy a halfway house between "positivism" — a commitment to the scientific method with its focus on providing uniform and reproducible results — and "hermeneutics" — the interpretative method of literary studies with their emphasis on differing points of view. As Mazlish sees it, the positivist impetus of the human sciences is constrained to coexist in an ever-unstable tension with the fragmenting impetus of diverse interpretative possibilities — hence the "uncertain" sciences of the book's title.

At this juncture, Mazlish turns to (one is tempted to say *into*) Georg Hegel. For he finds that this diversification and fragmentation will be resolved by history's gradual movement towards the whole, and thus an ever more comprehensive standpoint. "All we can say at this point is that a global scientific community appears essential for the human sciences."

The book's methodology becomes clear when one looks at its register of "major thinkers": William Whewell, Wilhelm Dilthey, Hans Blumenberg, Jürgen Habermas, Norbert Elias, Michael Foucault and Thomas Kuhn. (Otherwise, the individuals who rate more than three lines in the index are: Francis Bacon, Auguste Comte, the Marquis de Condorcet, Charles Darwin, René Descartes, Sigmund Freud, Hegel, Adam Smith, Anne-Robert-Jacques Turgot and Max Weber.) Clearly, Mazlish is focusing less on human sciences in the present day than on their history and the theories about them. This approach raises problems. After all, the world of learning is replete with non-Hegelian disciplines such as economics, sociology and psychology, where the history of thought on the issues will contribute little to settling the issues themselves.

Mazlish's discussion also touches too lightly on what is perhaps the most funda-

mental issue: whether the human sciences constitute a meaningful grouping at all. Just as the geologist, the landscape gardener, the painter and the military planner concern themselves with wholly different aspects of the same terrain, so too do those who study different aspects of man and his works. Each branch of inquiry has its own issues, concerns and methods. To believe that there is a distinctive sort of knowledge with which the human sciences can deal collectively is to beg some pretty large questions. They might constitute an externally unified area of discussion, but whether they constitute an internally coherent domain of inquiry can certainly be questioned. Otto von Bismarck is said to have remarked that Italy is not a nation but a geographic area for the convenience of cartographers. Analogously, it might be said that the human sciences are not a domain, but a taxonomic category for the convenience of librarians and academic administrators. Dealing with humans surely no more unites those various disciplines into a single domain than do botany, horticulture, cookery, agricultural economics and interior decoration constitute a single domain by the fact that they all deal with plants.

The human sciences exist all about us in splendid separation. And if it is science we want here, then we would do well to abandon the Hegelian universalism for a perhaps less inspiring but far more fruitful particularism. Science (rather like Satan) lies in the details. Thus, the question is not whether social psychology can predict the outcome of elections, but whether and to what extent public-opinion sampling can provide reliable election forecasts. Or again, not whether political theory provides an effective means of social control, but whether and to what extent advertising and product-promotion campaigns can increase sales. Just as it has been said that all politics is local, the same could be said of science in human affairs.

However, his Hegelianism leads Mazlish to insist on seeing the human sciences as a comprehensive and unified system rather than a mosaic of disciplines. He considers that the object of the enterprise is to provide a synoptic whole, a scientific treatment of human affairs in general. And now comes the big problem. Science requires consensus — one does not have a science unless its practitioners can develop a method that leads to generally acceptable solutions to the problems of the field. And this means that one is here hankering after the development of thought-systems that engender agreement on human affairs in general based on scientific principles. Accordingly, Mazlish is led to a deeply pessimistic conclusion: "There is little likelihood that the scientific community necessary for the human sciences will manifest itself in the near future ...

For the human sciences to develop, an average person will have to become knowledgeable about the human sciences."

Here, surely, we have a *reductio ad absurdum* of the author's rather grandiose view of the human sciences. For him, the answer to cultural relativism is to overcome the pluralism of different points of view and to look to the whole. But there is no reason to think that here — any more than elsewhere — looking at the same thing requires that it be looked at in the same way. There is good reason to think that, while we can settle matters of detail in the human sciences, it lies in the nature of things that consensus on most of the big questions is bound to elude us.

Mazlish's book is impressively learned and wide ranging in its information. It also offers many instructive observations and interesting insights. Nevertheless, it seems to be methodologically flawed, so that it fails to realize its goal of clarifying the sort of knowledge human sciences provide. □

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Non-sectarian structure

The Structure of Materials

by Samuel L. Allen and Edwin L. Thomas
Wiley: 1999. 447 pp. £32.50

Richard A. L. Jones

The strength of materials science arises from the way it crosses the boundaries between chemistry, physics and engineering. But if it has a weakness, it is a tendency to a tribalism based on classes of materials. Metals, ceramics and polymers at first sight look very different, and they are processed and used in very different ways. But the physical and chemical principles underlying their structure and behaviour are the same, even if an equation will often appear in two different guises as applied to, say, metals and polymers. So it is refreshing to see a textbook, written by a metallurgist and a polymer physicist, which sets out to discuss the structure of materials in an explicitly non-sectarian way.

In the past, a book on the structure of materials would have been dominated by crystallography. The treatment here is certainly quite complete, occupying a long, central chapter, but this book places an unusual strength of emphasis on other ways of describing structure. The emphasis on crystallography in materials science has in the past reflected the importance of materials that are fully crystalline, usually metals and ceramics. But important classes of materials are only partially crystalline, like some polymers, while others — liquid crystals and

glasses — are not crystalline at all. Structure in these materials must be described in other ways. For glasses we need pair-distribution functions, for liquid crystals the classification of mesophases and orientational order parameters, while for some disordered materials — soot, for example — the language of fractal geometry may be appropriate. These other types of description should also take their place in a textbook of materials structure. The result may be less appealing to tidy minds than a focus on pure crystallography, as these other descriptors vary greatly in their completeness and precision. But this is a fair reflection of the complexity of materials. After all, even in crystals, departures from perfect crystallinity, in the form of dislocations and point defects, can dominate properties, and it is quite right that this book takes almost as much space to discuss such defects as it devotes to the perfect crystal.

Where the book does follow materials science convention is in a rigid separation between the structure of materials and their dynamics. This is mostly for the best, but the separation does lead to some uncomfortable moments. For example, the distinction between a liquid and a glass is fundamentally one of dynamics rather than structure, though the authors attempt to make a structural distinction through the rather tricky notion of "free volume". By the final chapter, the distinction between structure and dynamics starts to blur; here an excellent dis-

cussion of structures on a hierarchy of lengthscales takes examples from polymers, composites and metals. Necessarily, this treatment must begin to discuss the kinetics of the phase changes that lead to these non-equilibrium states.

This is an attractive, and brief, book, which describes the structure of matter without artificial distinctions between different classes of materials. The point is made unobtrusively but nicely in the cover design. This shows two images, one a perfectly periodic array, which one might take to be a high-resolution lattice image of a crystal, and the other an attractive random pattern that a casual glance might mistake for an optical micrograph of a liquid crystal. Actually, the periodic pattern is a block-copolymer mesophase and the random pattern is an alloy defect texture. A textbook should bring out what different classes of materials have in common as well as identifying their differences; this book does that very well. □

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Also

Polymers at Surfaces and Interfaces

by Richard A. L. Jones & Randal W. Richards

Cambridge University Press, £29.95 (pbk)



Keeping it under wraps

It took the artists Christo and Jean-Claude 24 years to develop the "Wrapped Reichstag" project (shown above). Mountaineers worked to cover Berlin's Reichstag with 100,000 square metres of silvery polypropylene secured with 15,600 metres of blue rope. It was finished in

June 1995 and dismantled two weeks later. Christo rubs shoulders with fashion designers, textile artists, electroacoustic clothing and more in *Techno Textiles: Revolutionary Fabrics for Fashion and Design* by Sarah E. Braddock and Marie O'Mahony (Thames & Hudson, £16.95).

Why can't people be more like bats?

Cheating Monkeys and Citizen Bees: The Nature of Cooperation in Animals and Humans

by Lee Dugatkin

Free Press: 1999. 174 pp, \$25, £16.99

Laurent Excoffier

What have hermaphroditic sea bass, Mother Teresa and naked mole rats in common? This is not the latest dirty joke, but rather a serious issue treated in Lee Dugatkin's latest book *Cheating Monkeys and Citizen Bees*. These are three of the many organisms cited as displaying examples of cooperative behaviour, without which any society would, sooner or later, crumble. In order to avoid this fatal outcome, Dugatkin suggests, we should study the evolution of animal cooperation, because such a study "can be used to help us better understand and promote human cooperation". As animals present behaviours that are free of the influence of human culture, they can give us clues about cooperative strategies where moral will is absent, and where the only judge is natural selection.

Like four pillars of wisdom, Dugatkin describes four different strategies of cooperation: family dynamics, in which one is more likely to help relatives than strangers; reciprocal transactions, in which individuals cooperate with those they trust; selfish teamwork, in which cooperation can often be seen as a mere by-product of a selfish behaviour; and group altruism, in which individual altruistic behaviour (even though sacrificial) may be maintained when the unit of selection is the group where it belongs. This segmentation does not mean that animal cooperative behaviours are always so simple that they must necessarily belong to one of these categories, but these "four paths to cooperation" are based on sufficiently different evolutionary mechanisms to justify their distinction.

Each chapter is richly illustrated by well-chosen and often illuminating examples of cooperative behaviours, such as the baby-sitting behaviour of dwarf mongooses, the regurgitation of blood meals in vampire bats for their starving roostmates, the formation of coalition to rapt females in baboons, the exploratory tour of guppy fishes in front of predators, the helping behaviour of bee-eater birds towards their baby brothers and sisters, and the better-known social behaviours of ants, termite or bees. In all these cases, the author presents a very credible evolutionary explanation for the emergence and preservation of those behaviours by natural selection. Then, in an altruistic manner, he turns to the question of using this newly

acquired knowledge to foster cooperation in humans.

This is where the book goes off course. The potential applications of animal models of cooperation to humans are often appallingly simplistic, and mainly consist of a collection of recipes to improve the efficiency of teamwork. Police officers, soldiers, fire-fighters and work colleagues are the main groups he thinks could improve their cooperative behaviour. He suggests various measures to achieve this aim. Army or police units should be structured around relatedness, for example, since kin are more willing to help each other in dangerous situations. Small teams of related individuals would work harder because of kin-related benefits. Pairs of police officers should be teamed for long periods to increase their chances of interacting and helping each other. Reducing the size of teams could remove any temptation to cheat by working less.

As this book is targeted for a lay audience these ideas may well attract attention from people delighted (or afraid) to find some biological justification for nepotism or the creation of harsher working environments.

The book is a mixture of well-presented behavioural ecology and cheap sociology. Though Dugatkin is a recognized behavioural ecologist, he is far from being an outstanding philosopher. The human applications mentioned above almost all deal with people exposed to some sort of pressure (war, thieves, fire or work), where some penalty is imposed on cheats (death or a pay cut). The author's mistake is to transpose natural selection, a powerful explanatory paradigm in animals, into a mere coercive force in humans, implying that cooperation can be increased by additional constraints. Will the spirit of 1984 rule the twenty-first century? Probably, if we try to foster cooperation rather than harmony. □

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The Universe and everything

Cosmological Physics

by John A. Peacock

Cambridge University Press: 1999. 694 pp.

£70, \$85 (hbk); £24.95, \$39.95 (pbk)

Ray Carlberg

In the 1920s, a pair of discoveries — one observational and one theoretical — launched the modern era of cosmology. Hubble found his redshift distance relation, and Einstein's theory of general relativity was found to have either expanding or contracting solutions for a uniform, isotropic Universe.

By itself, either discovery would have been impressive. But the conjunction of a bold application of physical theory to the entire Universe and an observational fact was truly astonishing. There was plenty of immediate confusion, given the rather young Universe implied by Hubble's erroneous distance scale and a number of alternative physical models. More recently, a perhaps even deeper excitement has surrounded the application of new ideas in quantum-field theory and particle physics to the problems of the very early Universe and its relation to the existence of matter and structure. This new conjunction of physics and astrophysics offers tests of particle physics theory at energies far beyond any laboratory accelerator.

The intermingling of observational detail and fundamental theory has made cosmology an exceptionally rich, exciting and controversial science. Students in the field — whether observers or particle theorists — are expected to be acquainted with matters ranging from the Supernova Ia distance scale, Big Bang nucleosynthesis theory, scale-free quantum fluctuations during inflation, the galaxy two-point correlation function, particle theory candidates for the dark matter, and the star formation history of the Universe. Several general science books, conference proceedings and specialized monographs have addressed these issues. Peacock's *Cosmological Physics* ambitiously fills the void for introducing students with a strong undergraduate background in physics to the entire world of current physical cosmology. The majestic sweep of his discussion of this vast terrain is awesome, and is bound to capture the imagination of most students.

Cosmological Physics will appeal to the adventuresome, and may trouble those who like to see every detail of an argument spelled out. The avalanche of ideas rapidly, but not breathlessly, coaxes the reader straight into the excitement of the field. Peacock takes the view that an introduction to the physics of cosmology should not shield the reader from ideas and data whose significance is not yet clear. For instance, he includes brief sections on modified Newton dynamics and the anthropic principle. In spite of this, some of the observational details have already seen substantial changes in their interpretation, notably the redshift dependence of the star formation rate.

The result is an impressive overview of cosmology as a physical science. This abundance will communicate the widespread excitement of the subject as fundamental physics, and will inspire others to learn the details. □

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Evidence for lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima*

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The 1,860,725-base-pair genome of *Thermotoga maritima* MSB8 contains 1,877 predicted coding regions, 1,014 (54%) of which have functional assignments and 863 (46%) of which are of unknown function. Genome analysis reveals numerous pathways involved in degradation of sugars and plant polysaccharides, and 108 genes that have orthologues only in the genomes of other thermophilic Eubacteria and Archaea. Of the Eubacteria sequenced to date, *T. maritima* has the highest percentage (24%) of genes that are most similar to archaeal genes. Eighty-one archaeal-like genes are clustered in 15 regions of the *T. maritima* genome that range in size from 4 to 20 kilobases. Conservation of gene order between *T. maritima* and Archaea in many of the clustered regions suggests that lateral gene transfer may have occurred between thermophilic Eubacteria and Archaea.

Thermotoga maritima, a non-spore-forming, rod-shaped bacterium belonging to the order Thermotogales, was originally isolated from geothermal heated marine sediment at Vulcano, Italy¹, and has an optimum growth temperature of 80 °C. *T. maritima* metabolizes many simple and complex carbohydrates including glucose, sucrose, starch, cellulose and xylan^{1,2}. Both cellulose and xylan, through conversion to fuels (such as H₂), have great potential as renewable carbon and energy sources.

T. maritima is also of evolutionary significance, because small-subunit ribosomal RNA (SSU rRNA) phylogeny has placed this bacterium as one of the deepest and most slowly evolving lineages in the Eubacteria³. To elucidate further its unique metabolic properties and evolutionary relationship to other microbial species, we sequenced the genome of the type strain *T. maritima* MSB8 using the whole-genome random-sequencing method previously described^{4,5}.

General features of the genome

The genome of *T. maritima* is a single circular chromosome consisting of 1,860,725 base pairs (bp) (Fig. 1) with an average G + C content of 46%. A single rRNA operon (16S–23S–5S), containing an isoleucine transfer RNA and an alanyl tRNA in the spacer region between the small- and large-subunit genes, corresponds to the one region of the chromosome with a significantly higher G + C content (62%). A region of significantly lower G + C content (34%) encodes lipopolysaccharide biosynthesis (LPS) proteins.

On the basis of analysis of G + C ratio, G–C skew⁶ ($G - C/G + C$) and asymmetric distribution of oligomers⁷ in the *T. maritima* genome, we could not identify a characteristic bacterial origin of replication, a situation similar to that observed in the genomes of the Archaea *Methanococcus jannaschii*⁸ and *Archaeoglobus fulgidus*⁵. We assigned base-pair one of the genome at the beginning of the longest stretch (2.6-kb) of 30-bp repeats (Fig. 2).

Open reading frames. We identified 1,877 open reading frames (ORFs) (Figs 1, 2; Tables 1, 2), with an average size of 947

nucleotides, using the coding-analysis program GLIMMER⁹ (see Methods). Coding sequences cover 95% of the chromosome. Predicted protein sequences were searched against a non-redundant protein database and biological roles were assigned to 1,014 (54%)

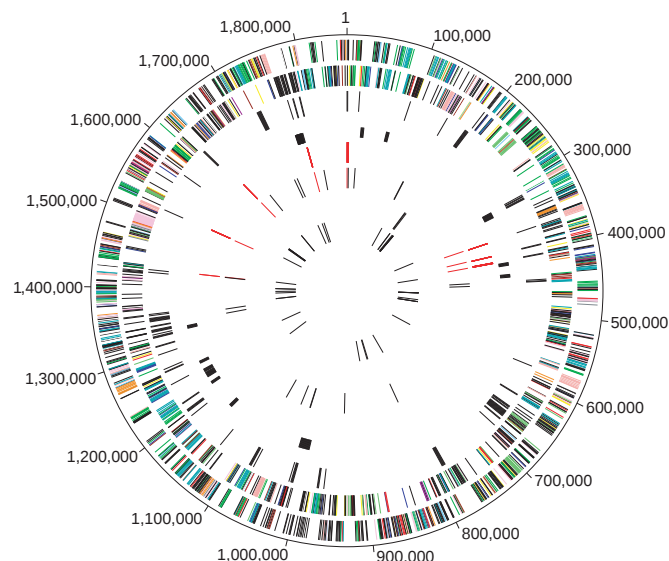


Figure 1 Circular representation of the *T. maritima* MSB8 genome showing predicted-coding regions and other features. Outer circle, predicted protein-coding regions on the plus strand classified by role according to the colour code in Fig. 2 (unknowns and hypotheticals are in black). Second circle, predicted protein-coding regions on the minus strand. Third circle, χ^2 composition in 2000-bp windows (see Methods); bands correspond to χ^2 values with $P \leq 1.9 \times 10^{-9}$. Fourth circle, Archaea-like islands on the genome. Fifth circle, small repeats. Sixth circle, large repeats (black), large repeats associated with small repeats (red). Seventh and eighth circles, rRNAs and tRNAs, respectively.

Table 1 General features of the *T. maritima* MSB8 genome

General features			
Length of sequence		1,860,725	
G + C ratio		46%	
Total no. of sequences		30,140	
Average read length (bp)		531	
Open reading frames		1,877	
Protein coding regions		95%	
Ribosomes		1 5S–16S–23S	
tRNAs		46 (10 clusters/19 single genes)	
Chromosomal coding sequences			
No. similar to known proteins		1,014	
No. of conserved hypotheticals		407	
No. similar to proteins of unknown function		83	
No. without a database match		373	
Total		1,877	
Repeats			
Class	Length	Copies	Database match
SR-01	30	143	tttcatacctctaaggaattattgaaaca
LR-01	1,897	2	hypothetical protein
LR-02	1,403	2	α -glucosidase
LR-03	1,137	4	putative transposase
LR-04	1,082	2	methyl-accepting chemotaxis protein
LR-05	858	2	putative transposase
LR-06	555	2	helicase
LR-07	252	2	exonuclease
LR-08	241	2	putative transposase

of them using the classification scheme adapted from ref. 10. Four-hundred-and-seven (22%) predicted coding sequences matched hypothetical coding sequences from other species, and 373 (20%) had no database match. Forty-six stable tRNAs with specificity for all 20 amino acids were identified.

Repeats. The *T. maritima* genome has 143 copies of a 30-bp repeat found in eight distinct clusters on the chromosome (Figs 1, 2). The 30-bp repeats are interspersed with a unique 39–40-bp sequence and are followed by a 452-bp sequence to form a structure identical to that described for the genomes of *M. jannaschii*⁸ and *A. fulgidus*⁵. Our examination of the *Aquifex aeolicus* genome¹¹ reveals that similar repeats are present, but they are fewer in number, and shorter, than those in *T. maritima*. These small/large repeat structures have only been identified in the genome sequences of thermophiles.

In addition to the 30-bp repeat structures, eight classes of large repeats, more than 200 bp in length and with >95% identity to each other, are present in the *T. maritima* genome (Fig. 1, Table 1).

Multigene families. We identified 214 gene families ($P \leq 10^{-5}$ over 60% of the length of the sequence—see Methods) in *T. maritima*. Of these families, 126 consist of two members, and the largest gene family (of ATP-binding subunits of ABC transporters) contains 67 members. Two other large families (one with 22 members and one with 47 members) consist exclusively of proteins involved in transport. Fifteen families, unique to *T. maritima*, consist of proteins with no database match. Eleven of these families have two members, and the largest group has seven members (<http://www.tigr.org/tdb/mdb/mdb.html>).

Solute uptake and metabolism

Several transporters reflecting the heterotrophic metabolism of this species are present, including those for importing maltose (*malE*), ribose (*rrsB*) and spermidine and/or putrescine (*potD*), as well as several carbohydrate transporters whose specificity is unknown. Carriers for the uptake of amino acids and oligopeptides, and ion-transport systems for the acquisition of K^+ (*trkA/H*), Mg^{2+} (*mgtE*), NH_4^+ (*amt*), PO_4^{2-} (*pstA/C/B/S*) and both oxidized and reduced forms of iron (*feoA/B*) are present. The large number of transporters for carbohydrates and amino acids (Figs 3, 4) suggests that the environment in which *T. maritima* is found is rich in organic material.

The predominant mechanism of transport in *T. maritima* is ATP-coupled solute flux. Eighty-four percent of the proteins in the transport category are subunits of ATP-binding cassette (ABC) transporters. Phylogenetic comparisons of the periplasmic solute-binding protein (SBP) component (Fig. 4) roughly parallels the families defined in other Eubacteria¹² with a marked expansion of proteins specific for oligopeptides. Nine of the eleven oligopeptide systems appear to be in operons with genes essential for sugar metabolism (Fig. 5). Of the published genomes, only *Pyrococcus horikoshii*¹³ has an oligopeptide transporter associated with a sugar degrading enzyme (β -galactosidase). This operonic structure suggests that there is coordinate regulation of peptide import with sugar degradation in these two organisms, although it is far more extensive in *T. maritima*. This contrasts with classical regulatory networks where the transported substrate affects transcription of the ABC transporter genes¹⁴.

Almost 7% of the predicted coding sequences in the *T. maritima* genome are involved in metabolism of simple and complex sugars, more than twice the percentage seen in other eubacterial and archaeal species sequenced to date. Several genes encoding proteins involved in the sequential degradation of xylan are present. Genes encoding endoglucanases (*celA/B*) and β -glucosidases (*bglB*) that are involved in cellulose degradation were identified, confirming the presence of the cellulolytic systems predicted by biochemical studies of this organism¹⁵. *T. maritima* does not have a complex system for the degradation of plant cellulosic materials, as described for the thermophilic bacterium *Clostridium thermocellum*, in which the degradation of cellulose depends on a multienzyme complex known as the cellulosome, composed of between 14 and 26 subunits¹⁶.

Glucose catabolism in *T. maritima* involves the Embden-Meyerhof and Entner-Doudoroff glycolytic pathways. In addition, the non-oxidative branch of the pentose-phosphate pathway appears to be involved in glucose breakdown. Genome analysis indicates that *T. maritima* can metabolize glycerol, gluconate and numerous sugars including amylose, maltose and galactose, as well as the amino acids aspartate, threonine and glycine (Fig. 3). CoA-SH-dependent ferredoxin oxidoreductases specific for pyruvate, as well as partial and complete operons for ferredoxin oxidoreductases of unknown specificity, are also present on the genome.

Biosynthetic pathways for nine amino acids were identified in *T. maritima* (Fig. 3). In addition, genes that encode proteins for the biosynthesis of biotin, folic acid, haem, porphyrin, lipoate, menaquinone, ubiquinone, pantothenate and pyridoxine were identified. *T. maritima* may also synthesize glycogen as a storage polysaccharide.

Along with an ability to gain energy through a fermentative metabolism, *T. maritima* can grow as a respiratory organism, generating energy in the presence of Fe(III) (ref. 17). Growth with sulphur as the terminal electron acceptor does not produce ATP¹⁸,

Figure 2 Linear representation of the *T. maritima* MSB8 genome. The locations of each predicted protein-coding region (colour-coded by biological role), RNA genes, tRNAs and repeat elements are indicated. Arrows represent the direction of transcription for each predicted coding region. Numbers next to the tRNA symbols represent the number of tRNAs at a locus. Numbers next to GES represent the number of membrane-spanning domains predicted by the Goldman, Engelman, and Steitz scale as calculated by TopPred⁴⁶ for that protein. Only proteins with five or more GES domains are shown. Presumed transporter specificity is indicated above predicted coding regions identified as transporters. Transporter abbreviations are as follows: +, cations; H⁺, protons; K⁺, potassium; Pi, phosphate; Zn, zinc; aa, amino acids; Na⁺, sodium; COH, sugar; aaX, oligopeptides; mal, maltose; rib, ribose; s/p, spermidine/putrescine; ura, uracil; ant, antibiotics; Fe²⁺, iron(II); Fe³⁺, iron(III); NH₄⁺, ammonium; bcaa, branched chain amino acids; g3Pi, glycerol-3-phosphate; glyc, glycerol; chro, chromate; Mg²⁺, magnesium; question marks (?) indicate where substrate specificity is uncertain or unknown. Members of paralogous gene families are identified by family number in a box above the predicted coding region.

but this pathway allows for the elimination of growth inhibitory H_2 which is produced during fermentative growth. Various flavoproteins and iron-sulphur proteins have been identified as potential electron carriers.

Response to environmental stimuli

T. maritima demonstrates a carbohydrate-dependent thermotactic response to temperature gradients between 50 and 105 °C (ref. 19).

Table 2 *T. maritima* MSB8 gene list

Gene identification numbers that correspond to those in Fig. 2 are listed here with the prefix TM followed by the common name assigned to each protein, the three- or four-letter gene name in parentheses, the organism with the most significant match in braces and the percent similarity to the best match. Each gene identified is listed in its functional role category (adopted from ref. 10). In cases where the substrate specificity of a protein could not be unambiguously determined, a more general common name was used and no gene name was assigned. In some cases a gene without known substrate specificity could be confidently assigned to a particular family as found in PROSITE (<http://expasy.hcuge.ch/sprot/prosite.html/>) or SWISS-PROT (<http://expasy.hcuge.ch/sprot/>). The term 'related' is used in two ways in the common names: (1) when the TM protein is a partial but significant match to a database protein: the TM protein is assigned to the Unknown role category; or (2) when the TM protein is a very good match to a database protein whose function is not found in *T. maritima*: the TM protein may be assigned to a role category appropriate to the known function of the database match. Abbreviations are as follows: Common names: AA, amino acid; NH₃, ammonia; NH₄⁺, ammonium; AFS, authentic frameshift; APM, authentic point mutation; Bprt, binding protein; BRAA, branched chain AA; Cl⁻, chloride; CoA, coenzyme A; DHase, dehydrogenase; dep, dependent; elong, elongation; fam, family; flgr, flagellar; init, initiation; Fe, iron; Fe³⁺, iron(III); Fe²⁺, iron(II); LPS, lipopolysaccharide; Mg²⁺, magnesium; Mn²⁺, manganese; OP, oligopeptide; PPase, phosphatase; P, phosphate; PPR, phosphoribosyl; K⁺, potassium; prt, protein; put, putative; RDase, reductase; reg, regulation, regulator, regulatory; rel, related; ssDNA, single stranded DNA; Na⁺, sodium; S, sulfur; sub, subunit; Sase, synthase, synthetase; term, termination; Tase, transferase; transp, transporter; Zn, zinc. Organism of best match: *Ac, Acinetobacter calcoaceticus*; *Ab, Agrobacterium bisporus*; *Ar, Agrobacterium radiobacter*; *At, Agrobacterium tumefaciens*; *Av, Alkaligenes eutrophus*; *Alc, Alkaligenes* sp.; *Alt, Alteromonas* sp.; *Amy, Amycolata* sp.; *Ana, Anabaena* sp.; *Ath, Anaerocellum thermophilum*; *Aa, Aquifex aeolicus*; *Atl, Arabidopsis thaliana*; *Af, Archaeoglobus fulgidus*; *Art, Arthrobacter* sp.; *Aca, Azorhizobium caulinodans*; *Av, Azotobacter vinelandii*; *Bbv, Bacillus brevis*; *Bca, Bacillus caldolyticus*; *Bce, Bacillus cereus*; *Bci, Bacillus circulans*; *Bf, Bacillus firmus*; *Bl, Bacillus licheniformis*; *Bm, Bacillus megaterium*; *Bac, Bacillus* sp.; *Bsp, Bacillus sphaericus*; *Bst, Bacillus stearothermophilus*; *Bs, Bacillus subtilis*; *T4, Bacteriophage T4*; *Bn, Bacteroides nodosus*; *Bt, Bacteroides thetaiotaomicron*; *Bv, Beta vulgaris*; *Bp, Bordetella pertussis*; *Bb, Borrelia burgdorferi*; *Bbr, Brevibacillus brevis*; *Bli, Brevibacterium linens*; *Ba, Brucella abortus*; *Ce, Caenorhabditis elegans*; *Cj, Campylobacter jejuni*; *Ca, Candida albicans*; *Ch, Capra hircus*; *Cc, Caulobacter crescentus*; *Cp, Chlamydia psittaci*; *Cr, Chlamydomonas reinhardtii*; *Cau, Chloroflexus aurantiacus*; *Cac, Clostridium acetobutylicum*; *Cla, Clostridium acididurici*; *Chi, Clostridium histolyticum*; *Cpa, Clostridium pasteurianum*; *Cpe, Clostridium perfringens*; *Clo, Clostridium* sp.; *Ct, Clostridium thermocellum*; *Cam, Corynebacterium ammoniagenes*; *Dd, Desulfovibrio desulfuricans*; *Df, Desulfovibrio fructosovorans*; *Dg, Desulfovibrio gigas*; *Dv, Desulfovibrio vulgaris*; *Dt, Dictyoglomus thermophilum*; *Eco, Eikenella corrodens*; *Ea, Enterobacter aerogenes*; *Ef, Enterococcus faecalis*; *Ech, Erwinia chrysanthemi*; *Ec, Escherichia coli*; *Eac, Eubacterium acidaminophilum*; *Eg, Euglena gracilis*; *Fi, Fervidobacterium islandicum*; *Hi, Haemophilus influenzae*; *Hp, Helicobacter pylori*; *Hs, Homo sapiens*; *Kp, Klebsiella pneumoniae*; *Lf, Lactobacillus fermentum*; *Lp, Lactobacillus pentosus*; *Li, Lactococcus lactis*; *Lpn, Legionella pneumophila*; *Lm, Leptospira meyeri*; *Lmo, Listeria monocytogenes*; *Mc, Mesembryanthemum crystallinum*; *Mta, Methanobacterium thermoautotrophicum*; *Mtf, Methanobacterium thermoformicum*; *Mj, Methanococcus jannaschii*; *Mk, Methylobacterium extorquens*; *Mlu, Micrococcus luteus*; *Ma, Microcystis aeruginosa*; *Mo, Micromonospora olivasterospora*; *Mmu, Mus musculus*; *Ml, Mycobacterium leprae*; *Ms, Mycobacterium smegmatis*; *Mt, Mycobacterium tuberculosis*; *Mg, Mycoplasma genitalium*; *Mp, Mycoplasma pneumoniae*; *Mx, Myxococcus xanthus*; *Nf, Naegleria fowleri*; *Ng, Neisseria gonorrhoeae*; *Nos, Nostoc* sp.; *Pd, Paracoccus denitrificans*; *Pha, Pasteurella haemolytica*; *Pan, Podospora anserina*; *Pg, Porphyromonas gingivalis*; *Pm, Propionigenium modestum*; *Pa, Pseudomonas aeruginosa*; *Pc, Pseudomonas cichorii*; *Pf, Pseudomonas fluorescens*; *Pp, Pseudomonas putida*; *Pse, Pseudomonas* sp.; *Ps, Pseudomonas stutzeri*; *Psy, Pseudomonas syringae*; *Pfu, Pyrococcus furiosus*; *Ph, Pyrococcus horikoshii*; *Pyr, Pyrococcus* sp.; *Rn, Rattus norvegicus*; *Ra, Reclinomonas americana*; *Rc, Rhodobacter capsulatus*; *Sc, Saccharomyces cerevisiae*; *Se, Saccharopolyspora erythraea*; *Sch, Salmonella choleraesuis*; *Sd, Shigella dysenteriae*; *Sa, Staphylococcus aureus*; *Sx, Staphylococcus xylosum*; *Sau, Stigmatella aurantiaca*; *Sb, Streptococcus bovis*; *Sm, Streptococcus mutans*; *Sp, Streptococcus pneumoniae*; *St, Streptococcus thermophilus*; *Scs, Streptomyces coelicolor*; *Ss, Sulfolobus solfataricus*; *Ssc, Sus scrofa*; *Syn, Synechococcus* sp.; *SPCC, Synechocystis PCC6803*; *Scy, Synechocystis* sp.; *Tb, Thermoaerobacter brockii*; *Tth, Thermoaerobacterium thermosaccharolyticum*; *Th, Thermococcus litoralis*; *Tm, Thermotoga maritima*; *Tn, Thermotoga neapolitana*; *Ta, Thermus aquaticus*; *Tt, Thermus thermophilus*; *Td, Treponema denticola*; *Tp, Treponema pallidum*; *Va, Vibrio alginolyticus*; *Vc, Vibrio cholerae*; *Vp, Vibrio parahaemolyticus*; *Ws, Wolinella succinogenes*; *Xl, Xenopus laevis*; *Ye, Yersinia enterocolitica*.

Motility is regulated by a two-component histidine kinase signal-transduction pathway that is assembled from products of the *Che* genes (*cheA/B/C/D/R/W/Y*) and seven methyl-accepting chemotactic transducer proteins (MCPs). Genes encoding *T. maritima* MCPs are most closely related to homologues in *Bacillus subtilis*, with specificity for both amino acids and carbohydrates. Although *T. maritima* demonstrates no chemotactic response to serine¹⁹, the presence of amino-acid-specific MCPs indicates that *T. maritima* may respond to aspartate, threonine and glycine, which appear to be catabolized by this bacterium.

In addition to the chemotactic-response kinases, other members of the two-component signalling family identified in *T. maritima* are likely to have a role in monitoring and responding to environmental stimuli such as temperature and nutrients. This group of six signalling partners includes the previously identified histidine-kinase (*hpkA*)/response-regulator (*rrrA*) pair belonging to the OmpR–PhoB subfamily of transcriptional regulators²⁰.

Although no heat- or cold-shock response has been experimentally demonstrated in *T. maritima*, the genome contains genes encoding heat-shock proteins (*dnaJ/K*, *groEL/ES*, *grpE*, *hslU/V* and *hsp*) and cold-shock proteins (encoded by *cspB/L*), which probably help regulate responses to changes in ambient temperature and growth conditions. *T. maritima* also contains genes encoding a general stress protein (*ctc*) and a stationary-phase-survival protein (*surE*), both of which are presumably involved in stress survival.

Cellular activities

Protein secretion, competence and transformation. In addition to the Sec-A-dependent secretory pathway, *T. maritima* has two specialized export systems. The first, which is assembled from homologues of FlhH/P/R and FlhA/B²¹, is probably associated with the secretion and assembly of the single *T. maritima* flagellum¹⁹. The second is a type-II secretion pathway that is assembled from the general secretion pathway proteins D, E and F (homologues of the PulD transport family of *Klebsiella oxytoca*²²). The *T. maritima* type-II secretion pathway probably serves as the primary mechanism for secretion of degradative enzymes required for the utilization of polysaccharides.

Competence has not been demonstrated in *T. maritima*, but it is possible that the type-II general secretion pathway proteins D, E and F, and the type IV pilin leader peptidase, type IV pilin-related protein and *pilT* identified in *T. maritima* may function in natural competence and transformation, as described for other organisms²³. In addition, *T. maritima* has homologues of the competence genes *dprA*, *comM* and *comE* of *Haemophilus influenzae*, and *comEA* and *comFC* of *B. subtilis*. There are other protein-coding sequences with weak homology to competence genes from *B. subtilis*. This suggests that there may be an inherent system for the uptake of exogenous DNA, facilitating genetic exchange between *T. maritima* and other organisms.

Transcription and translation. Genes encoding the three subunits (α , β , β') of the core RNA polymerase were identified in *T. maritima* (*rpoA/B/C*, respectively) along with four σ factors; σ^A (also named σ^{70}) (*rpoD*), σ^E (*rpoE*), σ^H (*fliA*) and σ^{28} (*spoOH*). Although σ^A has been previously identified in *T. maritima*²⁴, the roles and specificity of the remaining σ factors in transcription regulation are unknown. The *nusA/B/G* transcription antitermination genes and a member of the *greA/B* transcription-elongation-factor family were identified along with the *rho* transcription-termination factor.

The genome of *T. maritima* is similar to other sequenced bacterial genomes in that the gene for glutamyl tRNA-synthetase is missing. An alternative synthesis mechanism is the transamidation of Glu-tRNA^{Gln} to Gln-tRNA^{Gln} by Glu-tRNA^{Gln} amidotransferase, a heterotrimeric enzyme found in both Eubacteria and Archaea²⁵. Like *Helicobacter pylori*²⁶, *T. maritima* lacks an asparaginyl-tRNA synthetase. Transamidation of Asp-tRNA^{Asn} is presumably involved in generation of Asn-tRNA^{Asn}, similar to that reported in the archaeon *Haloferax volcanii*²⁷.

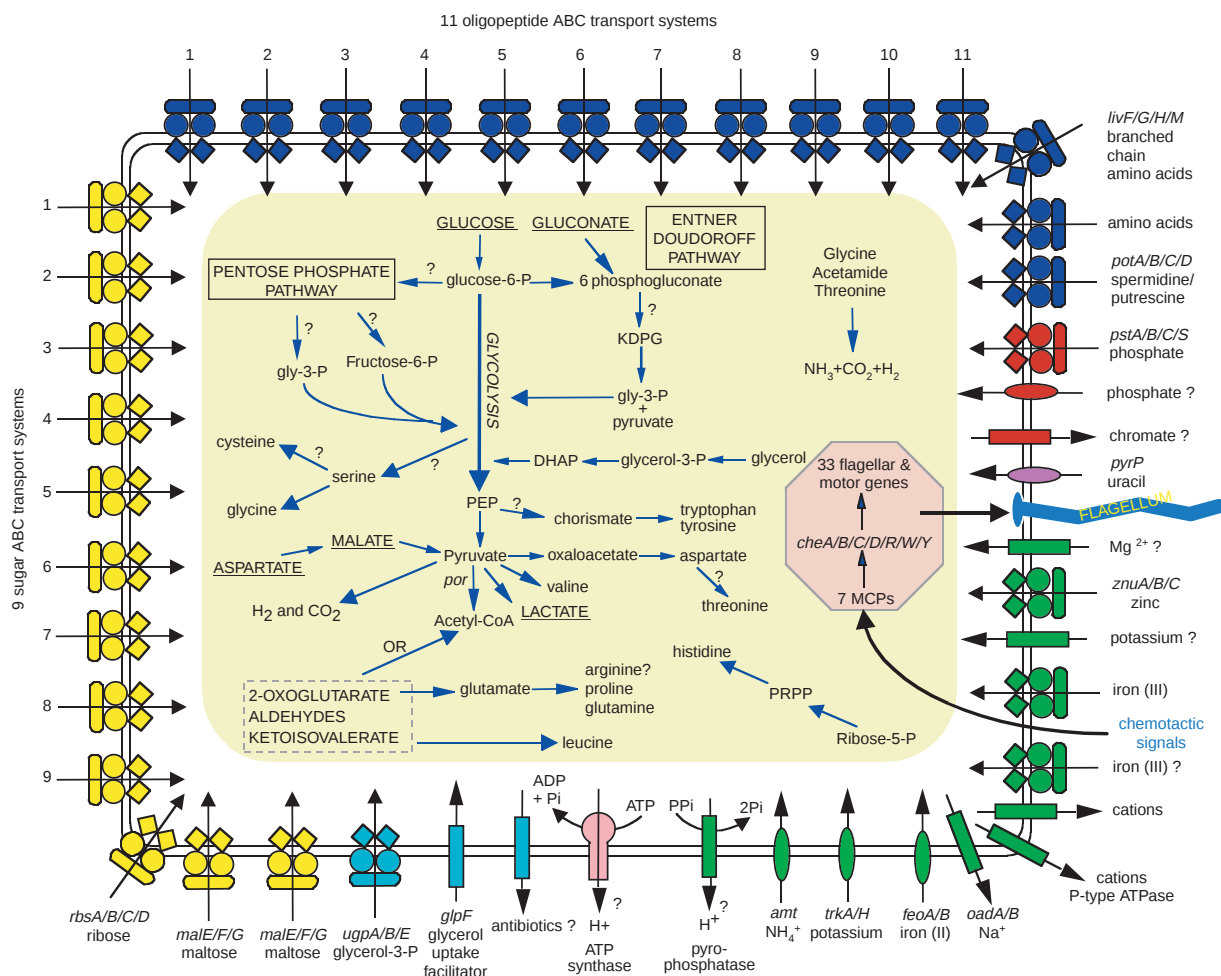


Figure 3 Overview of metabolism and transport in *T. maritima* MSB8. Pathways for energy production and the metabolism of organic compounds, acids and aldehydes are shown. Each gene product with a predicted function in ion or solute transport is illustrated. Transporters are grouped by substrate specificity according to role category: cations (green), anions (red), carbohydrates (yellow), purines (purple), amino acids/peptides/amines (dark blue) and other (light blue). Question marks associated with transporters indicate uncertainties in substrate specificity or direction of transport. Question marks associated with metabolic pathways indicate where an expected activity was not found. Permeases are represented by ovals. ABC transport systems are shown as

composite figures of ovals, diamonds and circles. All other transporters are drawn as rectangles. Export or import of solutes is designated by the direction of the arrows through the transporter. If precise substrate specificity could not be determined for a transporter, no gene name was assigned and a more general common name reflecting the type of substrate being transported was used. Abbreviations: PRPP, phosphoribosyl-pyrophosphate; gly, glyceraldehyde; PEP, phosphoenolpyruvate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; DHAP, dihydroxyacetone phosphate; OR, oxidoreductases; MCP, methyl-accepting chemotaxis protein; KDPG, 2-keto-3-deoxy-6-phosphogluconate; *por*, pyruvate oxidoreductase.

Cell division. The gene content of *T. maritima* demonstrates that the basic mechanism of cell division is similar to that found in other Eubacteria. The *ftsA/H/Y/Z* genes were identified along with two genes from the *min* locus, *minC/D*. These genes function together to specify the position and formation of the constricting ring that leads to final division.

Detoxification. Proteins for detoxification in this strict anaerobe include two NADH oxidases (*nox*), a putative alkyl hydroperoxide reductase, a heavy-metal-resistance transcriptional regulator and the periplasmic divalent cation-tolerance protein (*cutA*).

Phylogenetics and comparative genomics

The availability of the complete genome sequence of *T. maritima* is important for evolutionary studies, as *T. maritima* has been suggested to be one of the deepest branching eubacterial species, on the basis of phylogenetic analysis of SSU rRNA genes³. Although SSU rRNA analysis has been useful in identifying and characterizing bacterial strains and species, many questions remain regarding the validity of evolutionary conclusions based on SSU rRNA analysis (see, for example, ref. 28).

To capitalize on the information contained in completed genome sequences, and to reduce complications caused by different species sets in phylogenetic analyses of different genes²⁹, we identified a subset of 33 genes (see Methods) for which homologues were conserved in all species sequenced to date^{4,5,8,11,13,26,30–39} (Table 3). This subset was used to generate multiple sequence alignments and phylogenetic trees using both parsimony and distance methods (see Methods).

A few significant patterns arise from the analysis of these phylogenies. First, for the majority of genes, the Archaea constitute a distinct monophyletic group separate from the Eubacteria, a phylogenetic pattern also found in SSU rRNA trees. However, this finding does not relate to whether the Archaea as a whole are monophyletic, as the species of Archaea represented here are all *Euryarchaeota*. Second, most yeast genes group with the archaeal genes as found for SSU rRNA. In addition, in almost all trees, species that are part of the same bacterial phyla group together (for example, *Escherichia coli* and *H. influenzae*, *Borrelia burgdorferi* and *Treponema pallidum*). Beyond this, there are significant differences in the topologies of different genes, and there is little

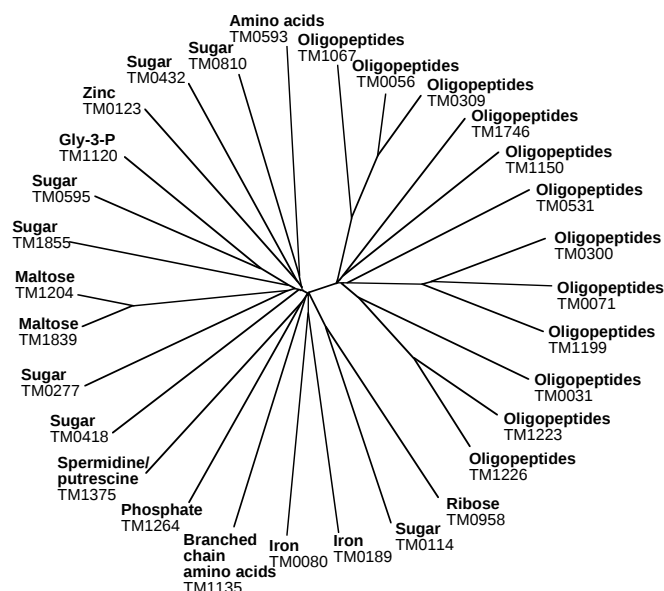


Figure 4 Phylogenetic pattern of the periplasmic SBP component of the oligopeptide transporters and other transporters present on the *T. maritima* MSB8 genome. Sperm/putre, spermidine/putrescine; Gly-3-P, glycerol-3-phosphate; sugar, unsure of specificity of sugar transporter.

agreement between these gene trees and the rRNA tree. In particular, we find little support for the rRNA-based positions of *Aquifex* and *Thermotoga*.

The lack of congruence of trees for different genes could result from the limited number of species represented by the completed genomes and/or the small size of some of these genes. However, we believe that the differences are real both because of high bootstrap support and because differences in topology have been found by others in the analysis of other genes²⁸. Mechanisms that could lead to these differences include gene duplication, gene loss and horizontal gene transfer. Thus we conclude that, based on single-gene analysis, the phylogenetic position of *Aquifex* and *Thermotoga*, and the nature of the deepest branching eubacterial species, should be considered to be ambiguous.

Phylogenetic analysis of individual genes has limited evolutionary studies to a small percentage of the genes in any given genome. As an alternative to single-gene phylogenetic analysis, we compared *T. maritima*'s genome sequence to those of the completely sequenced microbial species by observing patterns of similarity (see Methods). Of the 1,877 predicted coding sequences in the *T. maritima* genome, 52% are most similar to proteins in eubacterial species, most to the Gram-positive bacterium *B. subtilis* (21%) and to *A. aeolicus* (15%). In addition, 24% of the predicted coding sequences are most similar to proteins in archaeal species (Table 4),

almost half to *P. horikoshii*. This similarity of *T. maritima* to the Archaea contrasts with the other Eubacteria in which no more than 16% of the coding sequences in *A. aeolicus*, and 7% of the coding sequences in *B. subtilis*, are most similar to archaeal proteins. By whole-genome similarity comparison, *T. maritima* appears to be the most Archaea-like of all sequenced Eubacteria^{4,11,26,31–38}.

The Archaea-like nature of the *T. maritima* genome does not necessarily reflect a closely shared common ancestor between *T. maritima* and the Archaea, as the extensive similarity between these species could have arisen in many ways. These include loss of these genes in other lineages, extensive sequence divergence in mesophilic Eubacteria (coupled with a retention of such genes in the thermophilic Archaea and *T. maritima*) and, alternatively, that a few genes in the *T. maritima* genome with strong similarity to archaeal genes have expanded into large gene families, resulting in more genes with a higher level of similarity to archaeal genes. Such mechanisms could lead to similarity between Archaea and *T. maritima* regardless of the evolutionary history of these species.

We believe, however, that much of the similarity between *T. maritima* and the Archaea is due to shared ancestry of portions of the genome as a result of extensive lateral gene transfer between these lineages (Tables 3, 4). One line of evidence consistent with previous studies which have argued in support of lateral transfer⁴⁰ is that the 451 Archaea-like genes in *T. maritima* are not uniformly distributed among the biological role categories (Table 4). The majority of genes involved in housekeeping functions such as transcription, translation, DNA replication and cell division are most similar to orthologues in eubacterial species. In contrast, 49% of transporters (92), 60% of electron transport proteins (28) and 42% of conserved hypothetical proteins (173) are most similar to archaeal genes. Another observation which would support lateral gene transfer is that 81 of the Archaea-like genes in the *T. maritima* genome are clustered in 15 regions of the chromosome that range in size from ~4 to 20 kb. The genes, and conservation of gene order in seven of these regions, have only been described in the genome sequences of thermophilic Archaea. In addition, two of the clustered regions are associated with the 30-bp repeat elements found among Archaea and *T. maritima*, lending support to the idea that these repeat elements may be involved in gene transfer.

χ^2 -analysis of the genome sequence (see Methods) lends additional support to the theory of lateral gene transfer in *T. maritima*. Based on the assumption that the DNA composition is relatively uniform throughout the genome, there are at least 51 regions in the chromosome (Fig. 1) that have a significantly different composition ($P \leq 1.9 \times 10^{-9}$). Forty-two of these regions include genes and repeat structures that have highest levels of similarity to regions on the chromosomes of other thermophiles, including the thermophilic Archaea and *Aquifex*. All of the 30-bp small repeat areas have a χ^2 composition that is substantially different from the rest of the

Table 3 Genes shared by the *T. maritima* MSB8 genome

33 homologues in the completed genomes
apt, argS, eno, ftsZ, gcp, gltX, groES, hisT, pheS, pgk, prs rplA, rplB, rplC, rplE, rplF, rplK, rplN, rplR, rplV, rpsB, rpsC, rpsD, rpsE, rpsG, rpsH, rpsI, rpsK, rpsL, rpsM, rpsQ, rpsS, secY.

T. maritima genes matching only hyperthermophiles:

108 total; 93 hypothetical proteins; flgA putative; s-layer-related protein; rubrerythrin putative; 2 ABC transporters; glutamate synthase-related protein; glutaredoxin putative; putative glycerate kinase; putative hydrogenase; putative NADH dehydrogenase; putative LPS biosynthesis protein; putative phosphonopyruvate decarboxylase; putative pyruvate formate lyase activating enzyme; alanine acetyltransferase-related protein; sensory box protein.

T. maritima genes matching only Archaea

71 total; 64 hypothetical proteins; 2 ABC transporters; glutamate synthase-related protein; putative glycerate kinase; putative hydrogenase; rubrerythrin; sensory box protein.

Table 4 Top eubacterial or archaeal match in *T. maritima* by role ID

Role category	Eubacteria	Archaea	Eukaryotes	None
Amino acid biosynthesis	49	20	2	1
Purines, pyrimidines, etc.	32	11	2	0
Fatty acid and phospholipid metab.	12	3	0	0
Biosynthesis of cofactors etc.	22	10	0	0
Central intermediary metabolism	27	12	1	3
Autotrophic metabolism	0	0	0	0
Energy metabolism	117	56	1	18
Transport	89	92	0	7
DNA metabolism	45	6	0	2
Transcription	17	0	0	0
Translation	118	6	0	6
Regulatory functions	61	9	0	0
Cell envelope	57	9	1	7
Cellular processes	55	10	0	0
Other	9	8	0	1
Hypotheticals	213	173	2	19
Unknown	52	26	0	5
TOTAL	975	451	9	69

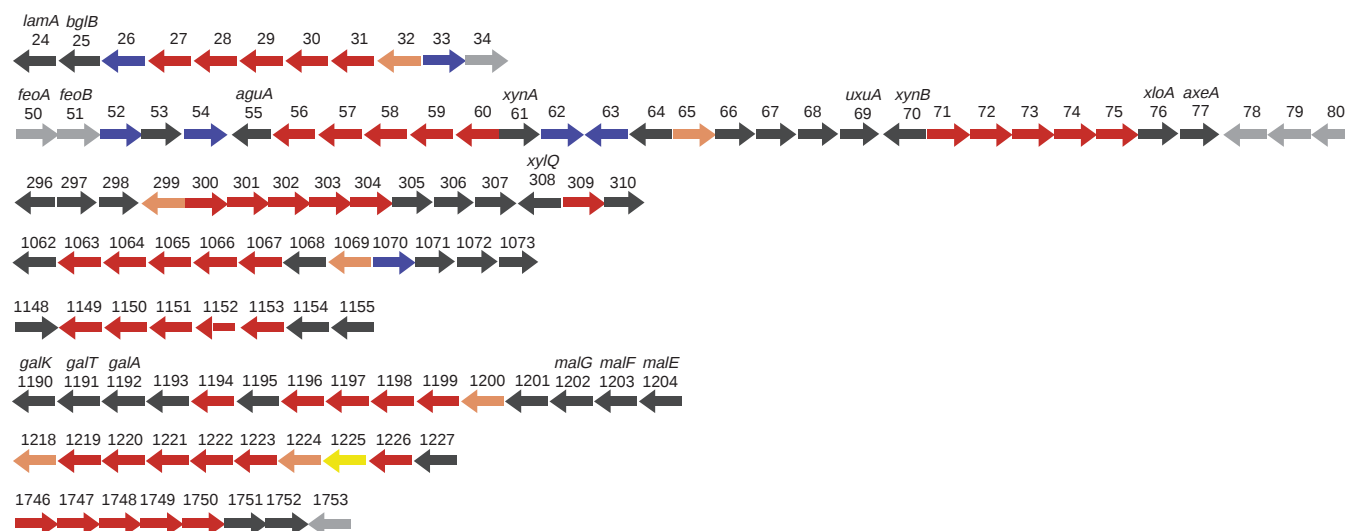


Figure 5 Linear representation of the location of nine oligopeptide transporter operons on the *T. maritima* MSB8 genome. Arrows represent functional categories as follows: black, sugar metabolism; red, transporters; orange, regulators; blue, hypothetical; yellow, conserved hypothetical; grey, other. *agaA*, α -glucuronidase; *axeA*, acetyl xylan esterase; *bglB*, β -glucosidase; *feoA*, iron(II)

genome. Compositional bias as seen in these χ^2 distributions has previously been used in support of lateral gene transfer⁴¹.

In summary, completion of the sequence of the *T. maritima* genome has revealed a degree of similarity with the Archaea in terms of gene content and overall genome organization that was not previously appreciated. Although the core of *T. maritima* may be eubacterial, almost one quarter of the genome is archaeal in nature. The mosaic nature of *T. maritima* appears to be the result of extensive lateral gene transfer with the Archaea. The accumulating evidence for the high frequency of lateral transfer events, and the lack of congruence among the phylogenies of different genes, indicates that the emphasis of phylogenetic studies on individual genes as indicators of organismal evolution is probably not accurate. Undoubtedly, our understanding of the complex relationships among prokaryotes will continue to increase as other microbial genome sequencing projects are completed. □

Methods

Whole-genome random sequencing procedure. The type strain, *T. maritima* MSB8, was grown from a culture derived from a single cell isolated by optical tweezers and provided by R. Huber (University of Regensburg). Cloning, sequencing and assembly were as described for genomes sequenced by TIGR⁴⁵. One small-insert plasmid library (1.5–2.5 kb) was generated by random mechanical shearing of genomic DNA. One large-insert lambda library was generated by partial *Tsp5091* digestion and ligation to λ -DASHIII/*EcoRI* vector (Stratagene). In the initial random sequencing phase, ~7-fold sequence coverage was achieved with 27,789 sequences from plasmid clones (average read length, 531 bases). The plasmid and λ -sequences were jointly assembled using TIGR Assembler⁴². Sequences from both ends of 546 λ -clones served as a genome scaffold, verifying the orientation, order and integrity of the contigs. Sequence gaps were closed by editing the ends of sequence traces and/or primer walking on plasmid clones. Physical gaps were closed by direct sequencing of genomic DNA, or combinatorial PCR followed by sequencing of the PCR product. The final genome sequence is based on 30,140 sequences.

Paralogous gene families were constructed by searching the ORFs against themselves using BLASTX⁴³, identifying pairwise matches above $P \leq 10^{-5}$ over 60% of the query search length, and subsequently clustering these matches into multigene families. Multiple sequence alignments for these protein families were generated using MSA (G. G. Sutton & T. Bussey, personal communication), an annealing algorithm, and the alignments were scrutinized.

transport protein A; *feoB*, iron(II) transport protein B; *galA*, α -galactosidase; *galK*, galactokinase; *galT*, galactose-1-phosphate uridylyltransferase; *lamA*, laminarinase; *malE/F/G*, maltose ABC transporter; *uxuA*, D-mannonate hydrolase; *xloA*, xylosidase; *xylQ*, α -xylosidase; *xynA*, endo-1,4- β -xylanase A; *xynB*, endo-1,4- β -xylanase B. Arrows are not proportional to the size of predicted-coding regions.

For the comparative genomics, the *T. maritima* ORFs were added to a set of all ORFs from 16 published microbial genomes^{4,5,8,11,13,26,30–39}. This dataset was searched against itself using BLASTX⁴³, and pair-wise matches were identified, clustered and converted to multiple sequence alignments as above. From this set of alignments a subset of 33 homologous gene families ($P \leq 10^{-5}$ over 60% of the query search length) were identified. These alignments were enhanced using the CLUSTAL X program, and regions of the alignments that were ambiguous, hypervariable or that contained large alignment gaps were excluded from subsequent phylogenetic analysis. Phylogenetic trees were generated from the curated alignments using the PAUP 4.0d64 and PHYLIP computer programs. Parsimony analysis was conducted using the heuristic search algorithm of PAUP and *protpars* of PHYLIP. Distance-based trees were generated using the neighbour-joining algorithm of ref. 44. One hundred bootstrap replicates were conducted for all trees. For all PAUP analyses, multiple step matrices were used in calculation of distances and in parsimony analysis. The whole genome set of pairwise BLASTX⁴³ search results was also used to determine 'best hits' of *T. maritima* and *A. aeolicus* to other genomes.

For the χ^2 analysis we computed the distribution of all 64 trinucleotides (3mers) for the complete genome, and then computed the 3mer distribution in 2,000 bp windows across the genome. We used windows that overlapped by half their length; that is, 1,000 bp. For each window, we computed the χ^2 statistic on the difference between its 3mer content and that of the whole genome. A large value of this statistic means that the composition within the window is different from the rest of the genome. Figure 1 illustrates those regions of the genome with χ^2 values whose probability is less than 1.9×10^{-9} ; the probability values for these regions are based on the assumption that the DNA composition is relatively uniform throughout the genome. Because this assumption may be incorrect, we prefer to interpret high χ^2 values merely as indicators of regions on the chromosome that appear unusual and that demand further scrutiny.

ORF prediction and gene family identification. An initial set of ORFs likely to encode proteins was identified by GLIMMER⁹, and those shorter than 30 codons were eliminated. ORFs that overlapped were visually inspected, and in some cases removed. ORFs were searched against a non-redundant protein database as previously described⁴. Frameshifts and point mutations were detected and corrected where appropriate as described previously²⁶. Remaining frameshifts and point mutations are considered to be authentic and corresponding regions were annotated as 'authentic frameshift' or 'authentic point mutation' respectively. Two sets of hidden Markov models (HMMs) were used to determine ORF membership in families and superfamilies. These included 527 HMMs from pfam v2.0, and 199 HMMs from the TIGR orthologue

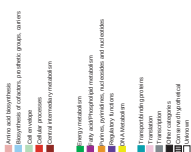
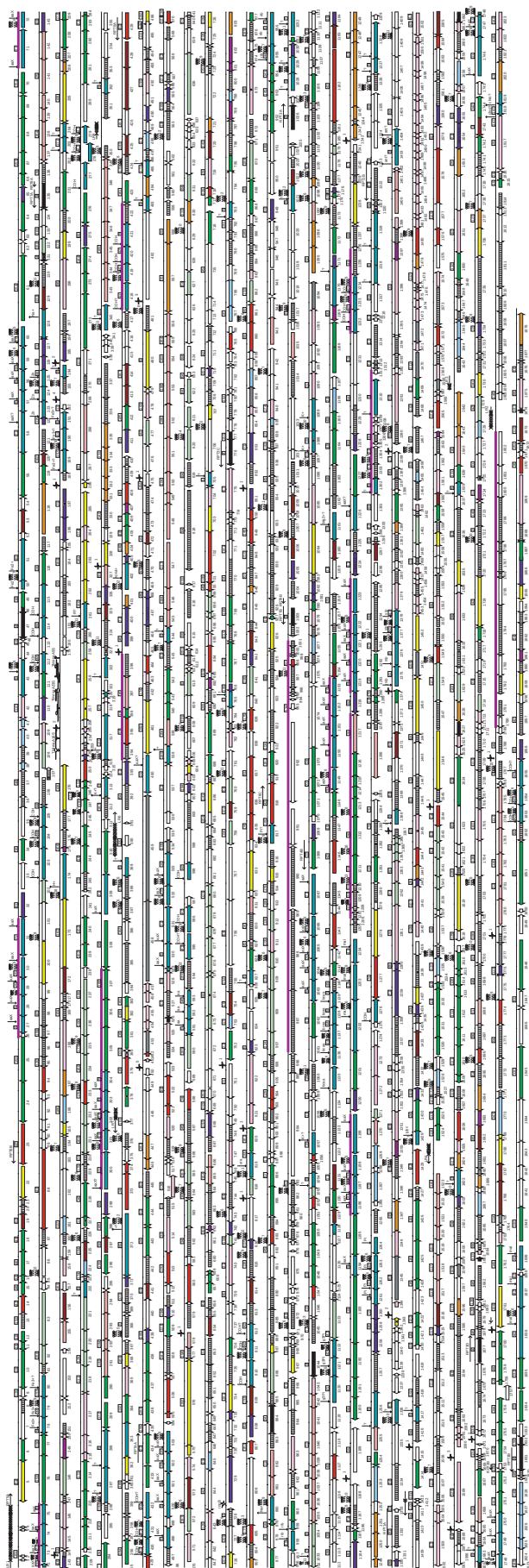
resource. TopPred⁴⁵ was used to identify membrane-spanning domains (MSDs) in proteins.

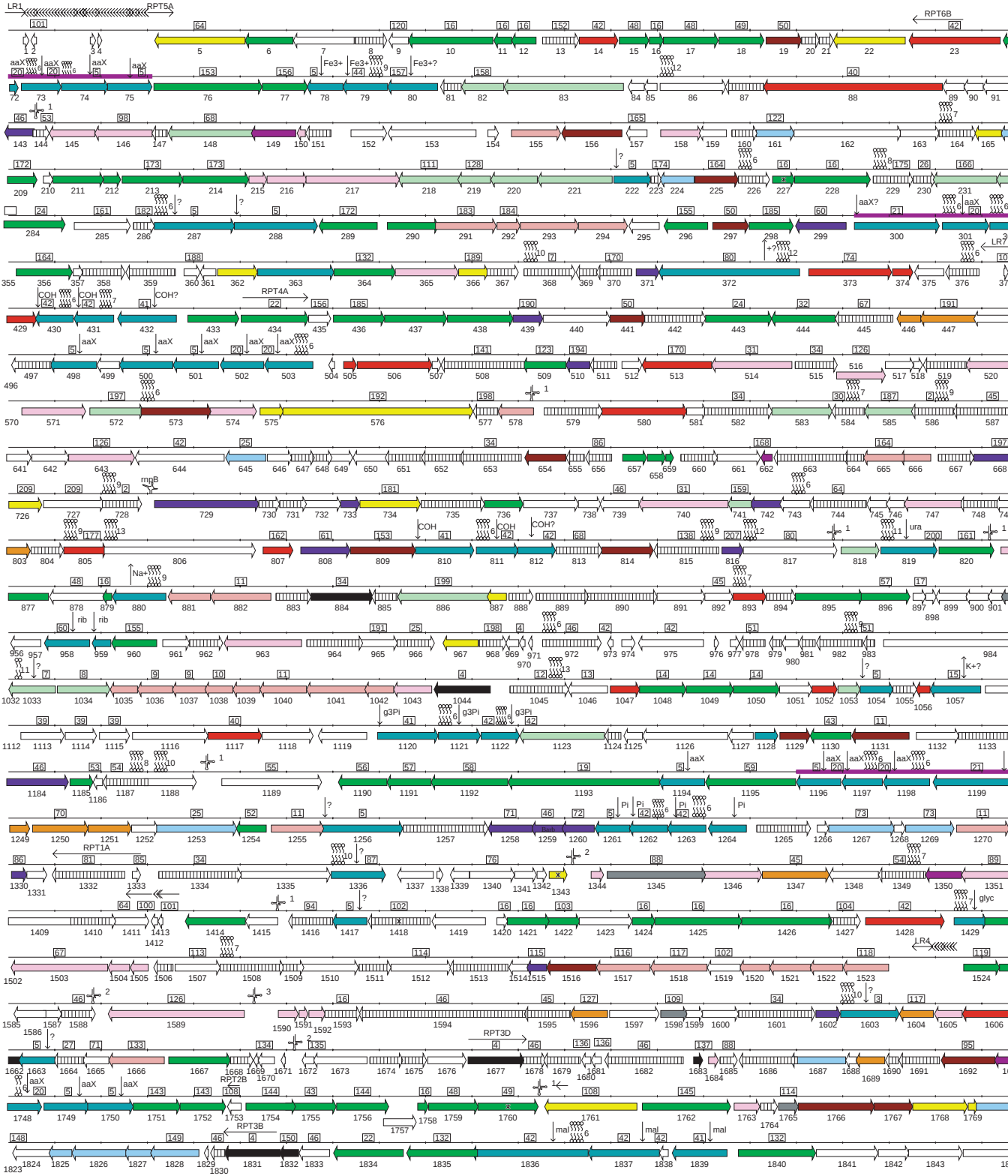
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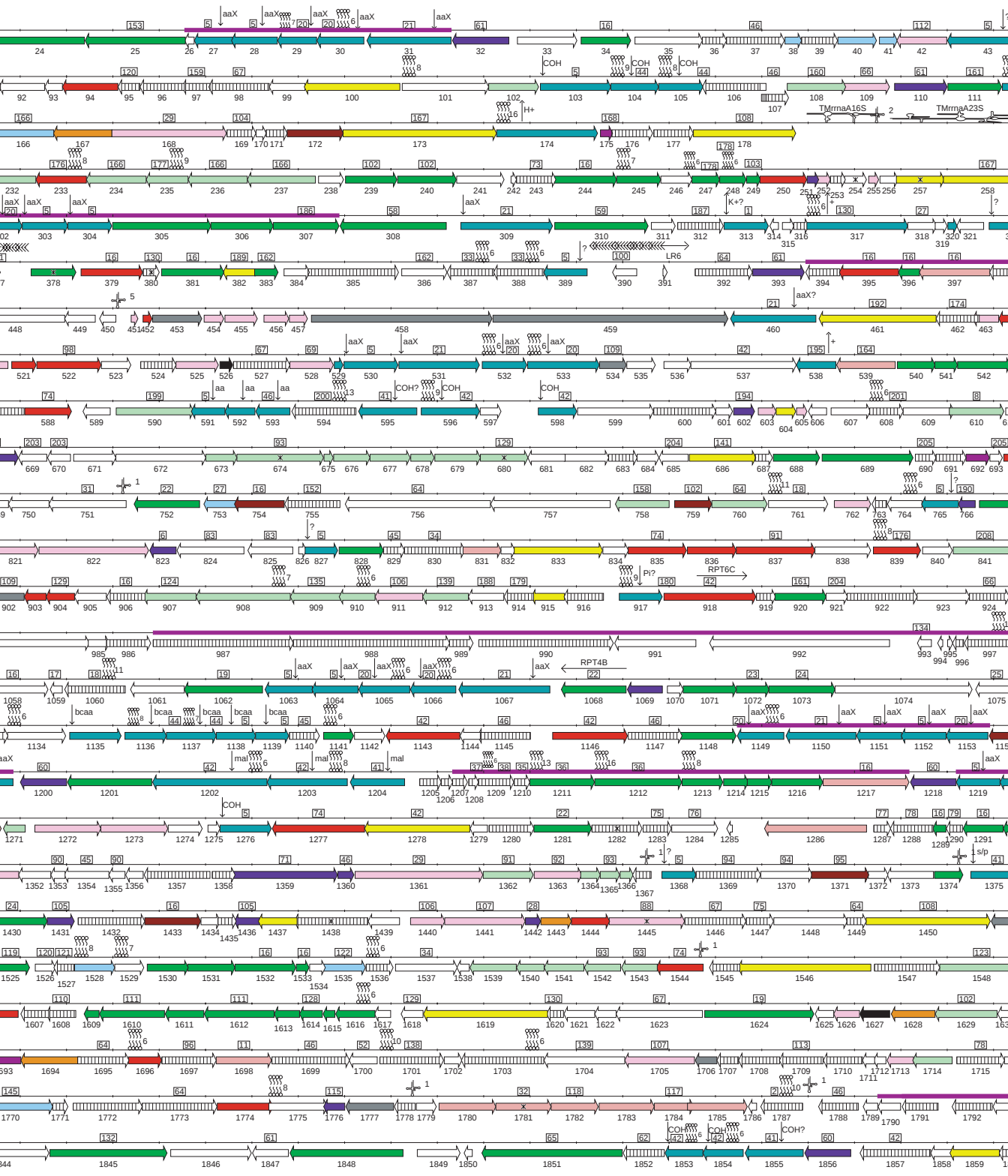
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Correspondence and requests for materials should be addressed to C.M.F. (e-mail: btm@tigr.org). The annotated genome sequence and the gene family alignments are available on the World-Wide Web at <http://www.tigr.org/tdb/mdb/>. The sequence has been deposited in GenBank with accession number AE000512.







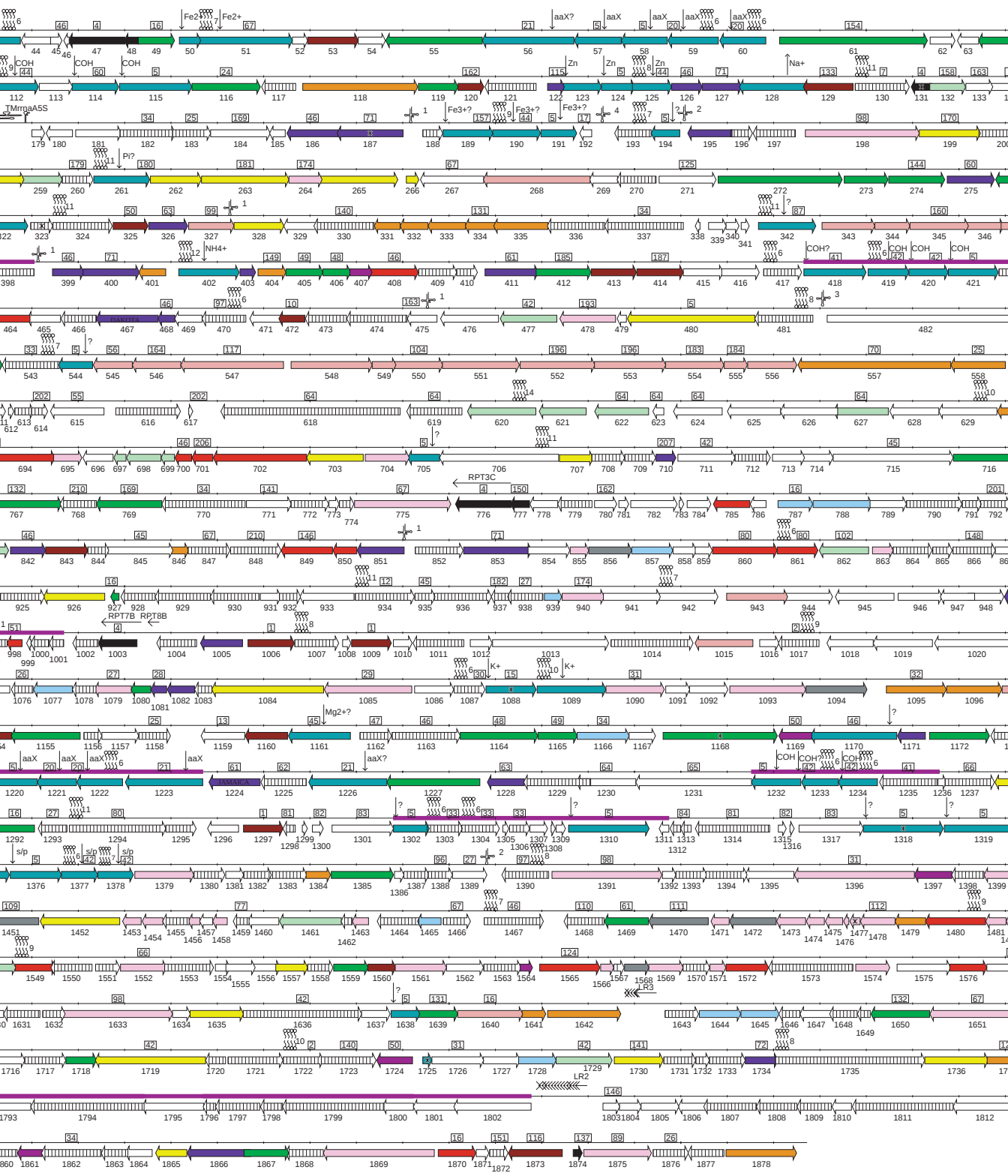


Table 2. *Thermotoga maritima* Gene List

Amino acid biosynthesis			TM1243	PPRaminimidazole-succinocarboxamide Sase (purC) [Mj]	68.0	TM1645	nicotinate-nucleotide pyrophosphorylase (nadC) [Mta]	62.1
<i>Aromatic amino acid family</i>								
TM0349	3-dehydroquinase dehydratase (aroD) [Hi]	70.7	TM1249	PPRaminimidazolecarboxamide formylase/IMP cyclohydrolase (purH) [Aa]	61.4	TM1644	quinolinate Sase A (nadA) [Mj]	70.1
TM0345	3-phosphoshikimate-1-carboxyvinylase (aroA) [Bs]	66.3	TM1251	PPRformylglycinamide cyclo-ligase (purM) [Hs]	58.5	<i>Other</i>		
TM0142	anthranilate Sase component I (trpE) [Tm]	99.8	TM1245	PPRformylglycinamide Sase I (purQ) [Bs]	71.8	TM0038	6-pyruvoyl tetrahydrobiopterin Sase, put [Ec]	62.2
TM0141	anthranilate Sase component II (trpGD) [Tm]	99.8	TM1246	PPRformylglycinamide Sase II (purL) [Aa]	62.4	TM0161	geranyltransase (ispA) [Bst]	62.1
TM0343	chorismate mutase, put [Sx]	73.2	TM1248	PPRglycinamide formylase (purN) [Bs]	70.6	TM1658	S-adenosylmethionine Sase (metK) [Bs]	81.8
TM0155	chorismate mutase/prephenate dehydratase [Af]	80.5	<i>Pyrimidine ribonucleotide biosynthesis</i>			Central intermediary metabolism		
TM0347	chorismate Sase (aroC) [Aa]	61.9	TM1642	aspartate carbamoylase, catalytic and reg chains (pyrBI) [Tm]	100	<i>Amino sugars</i>		
TM0140	indole-3-glycerol P Sase (trpC) [Tm]	99.6	TM0557	carbamoyl-P Sase, large chain (carB) [Af]	78.5	TM0814	N-acetylglucosamine-6-P deacetylase (nagA) [Bs]	56.6
TM0139	PPRanthranilate isomerase (trpF) [Tm]	100	TM0558	carbamoyl-P Sase, small chain (carA) [Aa]	75.3	<i>Polyamine biosynthesis</i>		
TM0344	prephenate DHase (tyrA) [SPCC]	49.4	TM0803	CTP Sase (pyrG) [Aa]	71.9	TM1873	ornithine decarboxylase (ode) [Aa]	64.2
TM0346	shikimate 5-DHase (aroE) [Aa]	66.9	TM0404	deoxycytidylate deaminase, put [Af]	74.0	TM0654	spermidine Sase (speE) [Bs]	69.7
TM0348	shikimate kinase/3-dehydroquinase Sase (aroBK) [SPCC]	54.8	TM0335	dihydroorotate (pyrC) [Bs]	55.2	<i>Other</i>		
TM0137	tryptophan Sase, alpha sub (trpA) [Tm]	100	TM0333	dihydroorotate DHase (pyrD) [Bs]	61.4	TM0225	1-aminocyclopropane-1-carboxylate deaminase, put [Ec]	55.1
TM0138	tryptophan Sase, beta sub (trpB-1) [Tm]	100	TM0334	dihydroorotate DHase electron transfer prt [Bs]	53.6	TM1129	5-methylthioadenosine/S-adenosylhomocysteine nucleosidase (pfs) [Bs]	55.8
TM0539	tryptophan Sase, beta sub (trpB-2) [Af]	77.0	TM0331	orotate PPRTase (pyrE) [Ta]	72.2	TM0573	acylase, put [Aa]	50.6
<i>Aspartate family</i>			TM0332	orotidine 5'-P decarboxylase, put [Ec]	52.2	TM0759	acylase, put [Mta]	63.4
TM0268	5-methyltetrahydrofolate S-homocysteine methylase [M]	54.3	TM0484	pyrimidine precursor biosynth enzyme, put [Sc]	52.2	TM0172	adenosylhomocysteine (ahcY) [Tm]	99.8
TM1286	5-methyltetrahydropteroyltrimethylglutamate—homocysteine methylase [Aa]	82.5	TM1694	thiamin biosynth prt ThiI (thiI) [Bs]	68.7	TM0156	alkaline PPase (phoB) [Bs]	58.5
TM1255	aspartate aminoTase (aspC-1) [Ta]	66.2	TM1099	thymidylate kinase (tmk) [Aa]	71.7	TM0472	amidoTase, put [M]	66.7
TM1698	aspartate aminoTase (aspC-2) [Ta]	53.3	TM1604	uridylylase kinase (pyrH) [Aa]	68.9	TM1371	aminoTase, class V (nifS) [SPCC]	61.4
TM1400	aspartate aminoTase, put [Mf]	66.3	<i>Salvage of nucleosides and nucleotides</i>			TM1692	aminoTase, class V (Ana)	62.0
TM1523	aspartate-semialdehyde DHase [Aa]	69.5	TM1384	adenine PPRTase (apt) [Bs]	75.3	TM1131	aminoTase, put [Ss]	64.2
TM1518	aspartokinase II (lysC-1) [Bst]	65.0	TM0846	cytidine/deoxycytidine deaminase (cdd) [Bs]	73.6	TM0129	carboxypeptidase G2, put [Pse]	53.1
TM0547	aspartokinase II (lysC-2) [Bst]	67.0	TM1443	cytidylate kinase (cmk) [Bs]	68.2	TM0413	creatinine amidohydrolase, put [Pse]	49.2
TM1270	cystathionine gamma-Sase (metB) [Hp]	59.5	TM1689	guanylate kinase (gmK) [Hp]	64.8	TM0414	DHase [Bs]	67.4
TM1517	diaminopimelate decarboxylase (lysA) [Aa]	66.6	TM0206	hypoxanthine PPRTase (hpt) [Bs]	66.7	TM1022	esterase (estA) [Tm]	100
TM1522	diaminopimelate epimerase (dapF) [Aa]	59.5	TM0167	phosphopentomutase (deoB) [Bs]	72.1	TM1160	esterase (estB) [Tm]	100
TM1520	dihydrodipicolinate RDase (dapB) [Psy]	62.7	TM1596	purine nucleoside phosphorylase (deoD-1) [Bs]	68.5	TM0053	esterase, put [Pa]	57.4
TM1521	dihydrodipicolinate Sase (dapA) [M]	72.5	TM1737	purine nucleoside phosphorylase (deoD-2) [Bs]	74.7	TM1766	formate—tetrahydrofolate ligase (fhs) [Cla]	74.1
TM0545	homoserine kinase, put [Aa]	61.8	TM1653	pyrimidine-nucleoside phosphorylase (deoA) [Bst]	74.5	TM0843	formiminoTase-cyclodeaminase/formimino-tetrahydrofolate cyclodeaminase, put [Ssc]	63.7
TM0881	homoserine O-succinylase (metA) [Ec]	69.8	TM0401	thymidine kinase (tdk) [Mp]	57.8	TM1585	glycerate kinase, put [Me]	55.6
TM1666	succinyl-diaminopimelate desuccinylase, put [Af]	71.2	TM0721	uracil PPRTase (upp) [Bca]	77.0	TM1516	hydrolase, ama/hipO/hyuC fam [Bs]	57.6
TM0546	threonine Sase (thrC) [Aa]	82.6	<i>Sugar-nucleotide biosynthesis and conversions</i>			TM0809	hydrolase, put [Alt]	59.6
<i>Glutamate family</i>			TM0630	nucleotide sugar epimerase, put [Mta]	74.9	TM1767	methylenetetrahydrofolate DHase/methylenetetrahydrofolate cyclohydrolase (folD) [Bs]	66.7
TM1784	acetylglutamate kinase (argB) [M]	74.8	TM1878	UDP-sugar hydrolase (ushA) [Ec]	63.0	TM0754	oxidoRDase [Ph]	63.4
TM1785	acetylornithine aminoTase (argD) [Aa]	71.6	Fatty acid and phospholipid metabolism			TM1743	oxidoRDase, aldo/keto RDase fam [Bs]	59.8
TM1781	argininosuccinate lyase (argH), AFS [Aa]	58.6	<i>Biosynthesis</i>			TM1009	oxidoRDase, aldo/keto RDase fam [Bs]	69.2
TM1780	argininosuccinate Sase (argG) [Mmu]	76.8	TM0801	(3R)-hydroxymyristoyl-(acyl carrier prt) dehydratase (fabZ) [Aa]	72.5	TM1006	oxidoRDase, aldo/keto RDase fam [Hp]	71.7
TM0293	gamma-glutamyl P RDase (proA) [Aa]	72.7	TM1693	1-acyl-sn-glycerol-3-P acetylase, put [Bb]	57.8	TM1183	oxidoRDase, aldo/keto RDase fam [Mta]	61.1
TM0294	glutamate 5-kinase (proB) [Aa]	64.9	TM1169	3-oxoacyl-(acyl carrier prt) RDase (fabG-1) [Aa]	61.7	TM0427	oxidoRDase, put [Aa]	45.1
TM1015	glutamate DHase [Tm]	100	TM11724	3-oxoacyl-(acyl carrier prt) RDase (fabG-2) [Bs]	71.8	TM1433	oxidoRDase, put [Af]	56.7
TM1783	glutamate N-acetylase (argJ) [Bst]	64.3	TM0802	3-oxoacyl-(acyl carrier prt) Sase II (fabF) [Aa]	68.7	TM0428	oxidoRDase, put [Af]	49.3
TM0397	glutamate Sase, alpha sub [Af]	81.2	TM0175	acyl carrier prt (acpP-1) [Aa]	69.1	TM1297	oxidoRDase, put [Bs]	55.8
TM1217	glutamate Sase, beta sub (glD-1) [Pyr]	58.5	TM0662	acyl carrier prt (acpP-2) [Aa]	86.3	TM0425	oxidoRDase, put [Bs]	48.1
TM1640	glutamate Sase, beta sub (glD-2) [Pyr]	74.8	TM1861	CDP-diacylglycerol—glycerol-3-P 3-phosphatidyl-Tase (pgsA) [Bs]	60.9	TM0120	oxidoRDase, put [Ce]	56.3
TM0943	glutamine Sase (glnA) [Tm]	99.5	TM0407	diacylglycerol kinase, put [SPCC]	58.3	TM0441	oxidoRDase, short chain DHase/RDase fam [Af]	62.9
TM1782	N-acetyl-gamma-glutamyl-P RDase (argC) [SPCC]	67.4	TM0149	fatty acylphospholipid synthesis prt (plsX) [Aa]	66.3	TM0019	oxidoRDase, short chain DHase/RDase fam [Bs]	59.4
TM1097	ornithine carbamoylase, anabolic (argF) [Tm]	100	TM0692	holo-(acyl carrier prt) Sase (acpS) [Ec]	63.5	TM0325	oxidoRDase, short chain DHase/RDase fam [Bs]	57.9
TM0578	pyrroline-5-carboxylate RDase (proC) [Aa]	60.2	TM0798	malonyl CoA-acyl carrier prt transacylase (fabD) [Bs]	64.2	TM0297	oxidoRDase, short chain DHase/RDase fam [Mt]	59.9
<i>Pyruvate family</i>			TM1397	phosphatidate cytidylase, put [Bs]	55.0	TM1154	oxidoRDase, sol/devB fam [Ana]	57.7
TM0553	2-isopropylmalate Sase (leuA) [Aa]	72.8	<i>Degradation</i>			TM1560	serine cycle enzyme, put [Me]	55.3
TM0552	2-isopropylmalate Sase, put [Aa]	81.1	TM1350	lipase, put [Af]	62.6	TM0720	serine hydroxymethylase (glyA) [Aa]	82.3
TM0554	3-isopropylmalate dehydratase, large sub (leuC) [Aa]	76.9	TM1564	acylPPase, put [Af]	69.7	Energy metabolism		
TM0291	3-isopropylmalate dehydratase, large sub, put [Af]	77.2	<i>Other</i>			<i>Amino acids and amines</i>		
TM0555	3-isopropylmalate dehydratase, small sub (leuD) [Af]	76.5	Biosynthesis of cofactors, prosthetic groups, and carriers			TM0119	acetamidase, put [Ms]	49.6
TM0292	3-isopropylmalate dehydratase, small sub, put [Af]	80.0	<i>Biotin</i>			TM0211	aminomethylase (gcvT) [Bs]	67.0
TM0556	3-isopropylmalate DHase (leuB) [Aa]	74.4	TM1269	biotin Sase, put [SPCC]	49.6	TM0444	aspartate NH3-lyase (aspA) [Bs]	64.1
TM0548	acetylactate Sase, large sub (ilvB) [Af]	71.8	TM0224	biotin—(acetyl-CoA carboxylase) Sase [Mta]	57.3	TM0212	glycine cleavage system H prt (gcvH) [Ec]	71.1
TM0549	acetylactate Sase, small sub (ilvN) [Af]	73.0	TM0799	bioY prt (bioY) [Bsp]	59.0	TM0213	glycine DHase (decarboxylating) sub 1 (yqhJ) [Aa]	69.3
TM0831	branched-chain AA aminoTase, put [Tm]	99.0	<i>Folic acid</i>			TM0214	glycine DHase (decarboxylating) sub 2 (yqhK) [Ph]	78.9
TM0551	dihydroxy-acid dehydratase (ilvD) [Aa]	74.0	TM0041	2-amino-4-hydroxy-6-hydroxymethyl-dihydropteridine pyrophosphokinase (folK) [Bs]	65.9	TM1744	L-allo-threonine aldolase [Ec]	67.6
TM0550	ketol-acid reductoisomerase (ilvC) [SPCC]	81.8	TM0040	dihydropterolate Sase (folP) [Bs]	68.6	TM0356	threonine dehydratase catabolic [Ec]	74.4
<i>Serine family</i>			TM0166	folylpolyglutamate Sase/dihydrofolate Sase (folC) [Bs]	57.8	<i>Anaerobic</i>		
TM0665	cysteine Sase (cysK) [Bs]	73.2	<i>Heme and porphyrin</i>			TM1867	L-lactate DHase (ldh) [Tm]	100
TM1401	D-3-phosphoglycerate DHase (serA) [M]	72.7	TM1465	cob(I)alamine adenosylase (cobA) [Ph]	72.8	<i>ATP-proton motive force interconversion</i>		
TM0882	O-acetylhomoserine sulfhydrylase (cysD) [Lm]	74.2	TM0488	hemK prt (hemK) [Hp]	62.4	TM1616	ATP Sase F0, sub a (atpB) [Bm]	60.9
TM0327	phosphoglycerate DHase, put [Ph]	68.8	TM1166	oxygen-independent coproporphyrinogen III oxidase, put [Aa]	62.1	TM1614	ATP Sase F0, sub b (atpF) [Bf]	68.7
TM0666	serine acetylase (cysE) [Sx]	59.9	<i>Menaquinone and ubiquinone</i>			TM1615	ATP Sase F0, sub c (atpE) [Eg]	67.2
<i>Histidine family</i>			TM1528	1,4-dihydroxy-2-naphthoate octaprenylase, put [Bs]	57.7	TM1612	ATP Sase F1, sub alpha (atpA) [Aa]	82.2
TM1038	amidoTase (hisH) [M]	64.2	TM1535	octoprenyl-diP Sase, put [Aa]	63.8	TM1610	ATP Sase F1, sub beta (atpD) [Tm]	100
TM1042	ATP PPRTase (hisG) [Aa]	60.8	TM0753	ubiquinone/menaquinone biosynth methylase, put [Bs]	54.8	TM1613	ATP Sase F1, sub delta (atpH) [Cr]	55.3
TM1036	cyclase (hisF) [Aa]	74.0	<i>Pantothenate</i>			TM1609	ATP Sase F1, sub epsilon (atpC) [Bf]	60.2
TM1041	histidinol DHase (hisD) [Bs]	68.5	TM1728	3-methyl-2-oxobutanoate hydroxymethylase (panB) [Ph]	75.7	TM1611	ATP Sase F1, sub gamma (atpG) [Aa]	58.5
TM1040	histidinol-P aminoTase (hisC) [Aa]	59.0	TM0939	aspartate 1-decarboxylase (panD) [Aa]	65.5	<i>Electron transport</i>		
TM1039	imidazoleglycerol-P dehydratase (hisB) [Ca]	56.1	TM1687	DNA/pantothenate metabolism flavoprt (dtp) [Aa]	65.0	TM1141	cytochrome C-type biogenesis prt, put [Hi]	56.1
TM1035	PPR-AMP cyclohydrolase / PPR-ATP pyrophosphohydrolase [Aa]	70.4	TM1077	pantoate—beta-alanine ligase (panC) [Aa]	74.2	TM1531	electron transfer flavoprt, alpha sub (etfA) [Cac]	68.2
TM1037	PPRformimino-5-aminoimidazole carboxamide ribotide isomerase (hisA) [L]	56.3	TM1825	6,7-dimethyl-8-ribityllumazine Sase (ribH) [Bs]	69.5	TM1530	electron transfer flavoprt, beta sub (etfB) [Cac]	66.5
Purines, pyrimidines, nucleosides, and nucleotides			TM1826	GTP cyclohydrolase II/3,4-dihydroxy-2-butanone 4-P Sase (ribA) [Bs]	63.1	TM1639	electron transfer prt, put [M]	57.6
<i>2'-Deoxyribonucleotide metabolism</i>			TM0857	riboflavin kinase/FMN adenylylase (ribF) [Aa]	59.9	TM0244	electron transport complex prt, put [Rc]	60.9
TM0118	ribonucleotide RDase, B12-dep (nrdJ) [Tm]	100	TM1827	riboflavin Sase, alpha chain (ribE) [Aa]	60.6	TM1426	Fe-hydrogenase, sub alpha (hydA) [Tm]	100
<i>Nucleotide and nucleoside interconversions</i>			TM1828	riboflavin-specific deaminase (ribD) [Aa]	65.5	TM1425	Fe-hydrogenase, sub beta (hydB) [Tm]	100
TM1479	adenylate kinase (ack) [Af]	74.9	<i>Thiamine</i>			TM1424	Fe-hydrogenase, sub gamma (hydC) [Tm]	100
<i>Purine ribonucleotide biosynthesis</i>			TM1770	1-deoxyxylulose 5-P Sase (dks) [Ec]	65.5	TM0927	ferredoxin (fdx) [Tm]	100
TM1095	adenylosuccinate lyase (purB) [Aa]	73.6	TM0788	thiamine biosynth prt, put [M]	75.1	TM1289	ferredoxin [Dd]	66.0
TM1096	adenylosuccinate Sase (purA) [Aa]	66.5	TM0787	thiamine biosynth enzyme [Mta]	67.8	TM1175	ferredoxin [M]	52.5
TM1247	amidoPPRTase (purF) [Bs]	66.1	TM1267	thiH prt, put [Ec]	53.9	TM1815	ferredoxin [M]	60.6
TM1641	dihydrofolate RDase (dyrA) [Tm]	100	TM1253	NH(3)-dep NAD(+) Sase (nadE) [Aa]	70.0	TM0879	ferredoxin [Mtp]	61.8
TM1820	GMP Sase (guaA) [Aa]	76.5	TM0645	NH(3)-dep NAD(+) Sase, put [M]	61.4	TM1533	ferredoxin (fixX) [Av]	68.8
TM1347	inosine-5'-monoP DHase (guaB) [Aa]	85.7	<i>Pyridine nucleotides</i>			TM1532	fixC prt (fixC) [Aca]	66.8
TM1628	PPR pyroP Sase (prs) [Cam]	75.9	TM1253	NH(3)-dep NAD(+) Sase (nadE) [Aa]	70.0	TM1031	glutaredoxin [Cpa]	73.6
TM1250	PPRamine—glycine ligase (purD) [Aa]	62.5	TM0645	NH(3)-dep NAD(+) Sase, put [M]	61.4	TM0868	glutaredoxin-rel prt (Pfu)	68.3
TM0447	PPRaminimidazole carboxylase, ATPase sub (purK) [Bs]	63.3				TM1421	hydrogenase, put [Dv]	52.2
TM0446	PPRaminimidazole carboxylase, catalytic sub (purE) [Mt]	73.6				TM1291	Fe-S cluster-Bprt [Af]	62.1

TM0880	oxaloacetate decarboxylase, beta sub (oadB) [Af]	74.0	TM0604	ssDNA-Bprt, put [Aa]	57.1	TM0863	ribosomal prt L9 (rplI) [Bst]	64.9
TM0174	pyroPPase, proton-translocating [Bv]	65.1	TM1546	ssDNA-specific exonuclease, put [Bs]	59.1	TM0456	ribosomal prt L10 (rplJ) [Tm]	99.4
TM1088	TRK system K+ uptake prt TrkA (trkA), AFS [Ec]	51.7	TM0726	tdld prt (tdld) [Ec]	60.2	TM0454	ribosomal prt L11 (rplK) [Tm]	100
TM1089	TRK system K+ uptake prt TrkH (trkH) [Mta]	56.9	TM1450	transcription-repair coupling factor, put [Bs]	64.7	TM1079	ribosomal prt L11 methylTase, put [Aa]	61.2
TM1725	vacuolar ATP Sase sub D-rel prt, APM [Ph]	83.7	<i>Restriction/modification</i>			TM1454	ribosomal prt L13 (rplM) [Aa]	83.0
TM0124	Zn ABC transp, ATP-Bprt (znuC) [Ec]	63.8	TM0328	m4C-methylTase [Hp]	58.4	TM1490	ribosomal prt L14 (rplN) [Tm]	100
TM0123	Zn ABC transp, periplasmic Zn-Bprt (znuA) [Ec]	49.6	<i>Degradation of DNA</i>			TM1481	ribosomal prt L15 (rplO) [Bst]	75.5
TM0125	Zn ABC transp, permease prt (znuB) [Ec]	53.9	TM1768	exodeoxyribonuclease VII, large sub (xseA) [Hi]	59.5	TM1493	ribosomal prt L16 (rplP) [Tm]	100
<i>Nucleosides, purines and pyrimidines</i>			TM1769	exodeoxyribonuclease, small sub (xseB) [Ec]	70.3	TM1471	ribosomal prt L17 (rplQ) [Hp]	65.0
TM0819	uracil permease (pyrF) [Bca]	69.4	TM1635	exonuclease, put [Aa]	55.1	TM1484	ribosomal prt L18 (rplR) [Aa]	84.6
<i>Other</i>			<i>Chromosome-associated proteins</i>			TM1571	ribosomal prt L19 (rplS) [Bst]	86.8
TM1403	antibiotic ABC transp, ATP-Bprt, put [Ph]	85.8	TM0266	DNA-Bprt, HU (hupB) [Tm]	98.9	TM1592	ribosomal prt L20 (rplT) [Bs]	69.6
TM1404	antibiotic ABC transp, transmembrane prt, put [Af]	77.9	Transcription			TM1458	ribosomal prt L21 (rplU) [Bs]	67.0
TM0363	fibronectin-Bprt, put [Bs]	54.0	<i>Degradation of RNA</i>			TM1495	ribosomal prt L22 (rplV) [Tm]	99.4
TM1429	glycerol uptake facilitator prt (glpF) [Bs]	79.9	TM1345	polynucleotide phosphorylase (pnp) [Bs]	70.0	TM1498	ribosomal prt L23 (rplW) [Tm]	100
TM1120	glycerol-3-P ABC transp, periplasmic glycerol-3-P-Bprt (ugpB) [Ec]	51.4	TM1102	ribonuclease III (rnc) [Bb]	65.2	TM1489	ribosomal prt L24 (rplX) [Tm]	100
TM1121	glycerol-3-P ABC transp, permease prt (ugpA) [Ec]	61.3	TM1472	DNA-directed RNA polymerase, alpha sub (rpoA) [Mt]	65.2	TM1456	ribosomal prt L27 (rpmA) [Ec]	75.0
TM1122	glycerol-3-P ABC transp, permease prt (ugpE) [Ec]	59.5	TM0458	DNA-directed RNA polymerase, beta sub (rpoB) [Tm]	100	TM0255	ribosomal prt L28 (rpmB) [Aa]	78.9
<i>Unknown substrate</i>			TM0459	DNA-directed RNA polymerase, beta' sub (rpoC) [Tm]	100	TM1492	ribosomal prt L29 (rpmC) [Tm]	100
TM1028	ABC transp, ATP-Bprt [Af]	60.5	<i>Transcription factors</i>			TM1482	ribosomal prt L30 (rpmD) [Bst]	75.0
TM0194	ABC transp, ATP-Bprt [Af]	61.2	TM1777	N utilization substance prt A (nusA) [Bs]	73.5	TM1684	ribosomal prt L31 (rpmE) [Tm]	100
TM0204	ABC transp, ATP-Bprt [Af]	68.3	TM1765	N utilization substance prt B (nusB) [Hi]	59.7	TM0150	ribosomal prt L32 (rpmF) [Aa]	72.4
TM0352	ABC transp, ATP-Bprt [Af]	79.5	TM0453	N utilization substance prt G (nusG) [Tm]	100	TM0451	ribosomal prt L32 (rpmG) [Tm]	100
TM0389	ABC transp, ATP-Bprt [Af]	65.9	TM0902	RNA polymerase sigma-28 factor, put [Lpn]	61.1	TM1591	ribosomal prt L35 (rpmI) [Bs]	65.6
TM0483	ABC transp, ATP-Bprt [Af]	66.3	TM1451	RNA polymerase sigma-A factor (rpoD) [Cac]	75.0	TM1476	ribosomal prt L36 (rpmJ) [SPCC]	81.6
TM0705	ABC transp, ATP-Bprt [Af]	73.6	TM1598	RNA polymerase sigma-E factor (rpoE) [Ec]	61.7	TM1445	ribosomal prt S1 (rpsA), AFS [Hi]	55.3
TM1327	ABC transp, ATP-Bprt [Bs]	64.2	TM0534	RNA polymerase sigma-H factor, put [Bl]	58.2	TM0762	ribosomal prt S2 (rpsB) [Bs]	81.4
TM0287	ABC transp, ATP-Bprt [Bs]	68.4	TM1706	transcription elong factor, greA/greB fam [Bs]	63.8	TM1494	ribosomal prt S3 (rpsC) [Tm]	100
TM0288	ABC transp, ATP-Bprt [Bs]	71.7	TM1470	transcription term factor Rho (rho) [Tm]	100	TM1473	ribosomal prt S4 (rpsD) [Hp]	69.9
TM1256	ABC transp, ATP-Bprt [Bs]	61.4	<i>RNA processing</i>			TM1483	ribosomal prt S5 (rpsE) [Bst]	76.9
TM1319	ABC transp, ATP-Bprt [Bs]	52.6	TM1568	16S rRNA processing prt, put [Ec]	60.6	TM0603	ribosomal prt S6 (rpsF) [Aa]	59.0
TM1328	ABC transp, ATP-Bprt [Bs]	53.4	TM1094	RNA methylTase, put [Bs]	57.5	TM1504	ribosomal prt S7 (rpsG) [Tm]	100
TM0043	ABC transp, ATP-Bprt [Bs]	59.4	TM0856	tRNA pseudouridine 55 Sase (truB) [Aa]	61.5	TM1486	ribosomal prt S8 (rpsH) [Bs]	78.8
TM1054	ABC transp, ATP-Bprt [Bb]	70.9	Translation			TM1453	ribosomal prt S9 (rpsI) [Bst]	75.0
TM1638	ABC transp, ATP-Bprt [Bb]	62.0	<i>tRNA aminoacylation</i>			TM1501	ribosomal prt S10 (rpsJ) [Tm]	100
TM1310	ABC transp, ATP-Bprt [Bbr]	50.8	TM1396	alanyl-tRNA Sase (alaS) [Aa]	69.0	TM1474	ribosomal prt S11 (rpsK) [Bac]	78.7
TM0222	ABC transp, ATP-Bprt [Mta]	65.0	TM1093	arginyl-tRNA Sase (argS) [Bs]	67.6	TM1505	ribosomal prt S12 (rpsL) [Aa]	95.2
TM1417	ABC transp, ATP-Bprt [Mj]	75.0	TM1441	aspartyl-tRNA Sase (aspS) [Aa]	75.0	TM1475	ribosomal prt S13 (rpsM) [Aa]	80.0
TM0765	ABC transp, ATP-Bprt [Mj]	61.4	TM0719	cysteinyl-tRNA Sase (cysS) [Aa]	69.3	TM1487	ribosomal prt S14 (rpsN) [Ta]	85.2
TM1663	ABC transp, ATP-Bprt [Mg]	63.5	TM1272	glutamyl-tRNA-Gln amidoTase, sub A (gatA) [Bs]	74.3	TM1344	ribosomal prt S15 (rpsO) [Bs]	78.7
TM1302	ABC transp, ATP-Bprt [Ph]	63.2	TM1273	glutamyl-tRNA-Gln amidoTase, sub B (gatB) [Aa]	75.2	TM1566	ribosomal prt S16 (rpsP) [Bs]	73.0
TM1368	ABC transp, ATP-Bprt [Ph]	70.8	TM0252	glutamyl-tRNA-Gln amidoTase, sub C (gatC) [Bs]	63.2	TM1491	ribosomal prt S17 (rpsQ) [Tm]	99.1
TM0544	ABC transp, ATP-Bprt [Ph]	64.4	TM1351	glutamyl-tRNA Sase (gltX-1) [Aa]	67.9	TM0605	ribosomal prt S18 (rpsR) [Bst]	75.0
TM0793	ABC transp, ATP-Bprt [Ss]	64.9	TM1875	glutamyl-tRNA Sase (gltX-2) [Aa]	65.4	TM1496	ribosomal prt S19 (rpsS) [Tm]	97.9
TM1318	ABC transp, ATP-Bprt, AFS [Bs]	53.9	TM0216	glycyl-tRNA Sase, alpha sub (glyQ) [Aa]	82.9	TM1657	ribosomal prt S20 (rpsT) [Bs]	61.2
TM0827	ABC transp, ATP-Bprt, put [Li]	57.1	TM0217	glycyl-tRNA Sase, beta sub (glyS) [Bs]	57.0	<i>tRNA modification</i>		
TM0322	ABC transp, periplasmic substrate-Bprt, put [Rc]	56.1	TM1090	histidyl-tRNA Sase (hisS) [Bs]	66.8	TM1574	pseudouridylylate Sase I (hisT) [Bs]	59.2
TM1170	ABC transp, periplasmic substrate-Bprt/conserved hypothetical prt [Ec]	46.3	TM1043	histidyl-tRNA Sase-rel prt [Bs]	50.8	TM1463	ribonuclease P prt component (mboA) [Pp]	65.6
TM0485	ABC transp, permease prt, cystTW fam [Hi]	63.4	TM1361	isoleucyl-tRNA Sase (ileS) [Tm]	100	TM0574	S-adenosylmethionine tRNA ribosylTase (queA) [Bs]	70.3
TM0203	ABC transp, permease prt, cystTW fam [Pa]	50.2	TM0168	leucyl-tRNA Sase (leuS) [SPCC]	69.1	TM0520	tRNA (5-methylaminomethyl-2-thiouridylylate)-methylTase (trmU) [Bs]	58.5
TM1029	ABC transp, permease prt, put [Bl]	50.3	TM1705	lysyl-tRNA Sase (lysS) [Sa]	72.7	TM0525	tRNA delta-2-isopentenylpyroP Tase (miaA) [Aa]	65.5
TM1032	permease, put [Bs]	48.5	TM0528	methionyl-tRNA formylTase (fmt) [Ec]	61.7	TM1561	tRNA guanine transglycosylase (tgt) [Bs]	69.9
TM1603	permease, put [Bs]	59.9	TM1085	methionyl-tRNA Sase (metS) [Tm]	100	TM1569	tRNA guanine-N1 methylTase (trmD) [Bs]	68.7
TM0342	permease, put [Ph]	66.0	TM0821	phenylalanyl-tRNA Sase, alpha sub (pheS) [Bs]	73.3	<i>Translation factors</i>		
TM1336	permease, put [Sp]	51.2	TM0822	phenylalanyl-tRNA Sase, beta sub (pheT) [Aa]	61.4	TM1363	peptide chain release factor RF-1 (prfA) [Aa]	75.6
TM1021	permease, put [Tm]	100	TM0514	prolyl-tRNA Sase (proS) [Aa]	69.6	TM1579	peptide chain release factor RF-2 (prfB) [Aa]	78.3
DNA metabolism			TM1379	seryl-tRNA Sase (serS) [Aa]	77.1	TM1399	ribosome recycling factor (rrf) [Bs]	69.6
<i>DNA replication, recombination, and repair</i>			TM0740	threonyl-tRNA Sase (thrS) [Bs]	73.5	TM1503	translation elong factor G (fus-1) [Tm]	99.1
TM0205	ATP-dep DNA helicase (recG) [Bs]	63.6	TM0492	tyrosophanyl-tRNA Sase (trpS) [Bb]	62.8	TM1651	translation elong factor G (fus-2) [Aa]	66.4
TM1238	ATP-dep DNA helicase (uvrD) [Ti]	58.6	TM0478	tyrosyl-tRNA Sase (tyrS) [Aa]	76.9	TM1763	translation elong factor P (efp) [Mt]	63.2
TM0926	chromosomal replication initiator prt (dnaA) [Tm]	100	TM1817	valyl-tRNA Sase (valS) [Bst]	72.4	TM1605	translation elong factor Ts (tsf) [Ta]	75.6
TM0703	competence-damage inducible prt, put [Bs]	61.6	<i>Degradation of proteins, peptides, and glycopeptides</i>			TM1502	translation elong factor Tu (tuf) [Tm]	99.8
TM0575	crossover junction endodeoxyribonuclease (ruvC) [Hi]	63.3	TM0042	aminopeptidase P, put [Li]	63.6	TM1477	translation init factor IF-1 (infA), AFS [Bs]	84.9
TM0362	deoxyribonuclease IV (nfo) [Aa]	58.7	TM0365	aminopeptidase, put [Bb]	69.1	TM0775	translation init factor IF-2 (infB) [Aa]	69.8
TM1437	dimethyladenosine Tase (ksgA) [Bs]	64.0	TM0198	ATP-dep Clp protease, ATPase sub (clpC-1) [Scy]	74.4	TM1590	translation init factor IF-3 (infC) [Aa]	74.4
TM1084	DNA gyrase, sub A (gyrA) [Tm]	99.9	TM0873	ATP-dep Clp protease, ATPase sub (clpC-2), AFS [Scy]	74.0	TM0911	translation init factor, aIF-2B alpha sub-rel [Af]	70.7
TM0833	DNA gyrase, sub B (gyrB) [Tm]	100	TM1391	ATP-dep Clp protease, ATPase sub (clpC-3) [Scy]	74.0	TM1440	translation init factor, eIF-2B alpha sub-rel [Rn]	53.0
TM0005	DNA helicase, put [Af]	77.6	TM0146	ATP-dep Clp protease, ATPase sub clpX [Ec]	81.7	<i>Other</i>		
TM0100	DNA ligase (ligA) [Aa]	68.8	TM0695	ATP-dep Clp protease, proteolytic sub (clpP) [Aa]	86.1	TM1626	peptidyl-tRNA hydrolase (SPCC)	62.4
TM0022	DNA mismatch repair prt (mutL) [Tm]	100	TM1633	ATP-dep protease LA (lon) [Bbv]	71.9	TM0215	prt synthesis inhibitor, put [Bs]	71.8
TM1719	DNA mismatch repair prt (mutS) [Tm]	99.5	TM1869	ATP-dep protease LA, put [Hi]	55.6	TM0855	ribosome binding factor A (Sau)	57.5
TM1278	DNA mismatch repair prt, put [Bs]	60.3	TM0747	carboxyl-terminal protease [Aa]	62.6	Regulatory functions		
TM0576	DNA polymerase III, alpha sub (dnaE) [Bs]	68.9	TM1589	clostripain-rel prt [Chi]	50.3	TM0729	(p)ppGpp Sase (relA) [Bs]	64.5
TM0461	DNA polymerase III, alpha sub, put [Aa]	59.8	TM0516	clostripain-rel prt [Chi]	50.3	TM1081	anti-sigma factor antagonist, put [Bl]	64.2
TM0262	DNA polymerase III, beta sub (dnaN) [Hi]	54.7	TM0643	clostripain-rel prt [Chi]	49.0	TM1442	anti-sigma factor antagonist, put [Bl]	53.3
TM0496	DNA polymerase III, epsilon sub, put [Aa]	55.6	TM1823	ftsH protease activity modulator HflC (hflC) [Vp]	62.0	TM0371	arginine repressor (argR) [Bs]	62.0
TM0686	DNA polymerase III, gamma and tau sub (dnaZX) [Bs]	57.9	TM1822	ftsH protease activity modulator HflK (hflK) [Vp]	58.4	TM0251	carbon storage reg (csrA) [Ec]	78.3
TM1452	DNA primase (dnaG) [Aa]	58.5	TM0571	heat shock serine protease, periplasmic (htsrA) [Ba]	61.1	TM0122	ferric uptake reg prt (fur-1) [Cj]	57.1
TM0199	DNA repair prt (radA) [Ec]	63.8	TM0963	oligoendopeptidase, put [Bb]	49.9	TM1515	ferric uptake reg prt (fur-2) [SPCC]	59.7
TM1557	DNA repair prt (radC) [Bs]	66.8	TM1346	processing protease, put [Bs]	51.1	TM1776	ferric uptake reg prt (fur-3) [Ng]	56.5
TM1859	DNA repair prt (recA) [Tm]	100	TM1713	proline dipeptidase, put [Bb]	59.2	TM1431	glycerol uptake operon antiterminator (glpP) [Bi]	61.1
TM0257	DNA replication enhancer, put, AFS [Fi]	76.9	TM0145	secreted metalloendopeptidase Gcp, put [Pha]	65.8	TM1436	glycerol uptake operon antiterminator-rel prt [Bs]	55.2
TM0258	DNA topoisomerase (topA) [Tm]	100	<i>Protein modification</i>			TM0195	guanosine pentaP phosphohydrolase, put [Hp]	49.6
TM1619	DNA-directed DNA polymerase I (polA) [Cau]	65.5	TM0704	L-isoaspartate(D-aspartate) O-methylTase, put [Tm]	100	TM0851	heat shock operon repressor HrcA [Bs]	53.5
TM0366	endonuclease III (nth) [Mta]	74.7	TM0463	lipopt signal peptidase [Aa]	61.2	TM0510	Fe-dep transcriptional repressor, put [Mt]	62.6
TM1865	endonuclease V (nfi) [Ec]	65.9	TM1478	methionine aminopeptidase (map) [Bs]	69.4	TM0602	Fe-dep transcriptional repressor, put [Mt]	45.0
TM0480	excinuclease ABC, sub A (uvrA) [Aa]	77.8	TM1661	polypeptide deformylase (def) [Tm]	100	TM1330	laci fam transcriptional reg, put [Mj]	70.4
TM1761	excinuclease ABC, sub B (uvrB) [Mta]	74.7	TM0158	poliropot diacylglycerol Tase (lgt) [SPCC]	68.0	TM1082	lexA repressor (lexA) [Tm]	100
TM0265	excinuclease ABC, sub C (uvrC) [Mta]	60.9	TM0109	pyruvate formate lyase activating enzyme, put [Ti]	59.0	TM1866	membrane bound prt LytR, put [Bs]	52.3
TM0734	glucose-inhibited division prt (gidJ) [Bs]	72.1	TM1552	pyruvate formate-lyase activating enzyme, put [Af]	68.0	TM0403	nitrogen reg prt P-II (glnK) [Av]	69.6
TM0263	glucose-inhibited division prt A (gidA) [Bs]	72.0	<i>Ribosomal proteins: synthesis and modification</i>			TM1259	P regulon transcriptional reg prt PhoB (phoB) [Sdj]	63.6
TM0707	glucose-inhibited division prt B (gidB) [Pp]	58.9	TM0264	16S pseudouridylylate Sase (rsuA) [Ec]	59.8	TM1260	P transport system reg PhoU (phoU) [Pa]	55.1
TM0165	Holliday junction DNA helicase (ruvA) [Ec]	50.8	TM0940	ribosomal large sub pseudouridine Sase C (rluC) [Ec]	57.7	TM1734	P transport system reg PhoU, put [Aa]	57.7
TM1730	Holliday junction DNA helicase (ruvB) [Tm]	100	TM0455	ribosomal prt L1 (rplA) [Tm]	100	TM1184	pleD-rel prt [SPCC]	70.8
TM0967	integrase-recombinase prt (xerC) [Mta]	63.5	TM1497	ribosomal prt L2 (rplB) [Tm]	99.3	TM0668	pleiotropic reg prt (degT) [Bst]	54.5
TM0887	methylated-DNA-prt-cysteine methylTase (ogt) [Af]	64.7	TM1500	ribosomal prt L3 (rplC) [Tm]	100	TM0467	reg prt, put [SPCC]	54.4
TM0178	primosomal prt N' (priA) [Bs]	54.7	TM1488	ribosomal prt L4 (rplD) [Tm]	99.1	TM0490	reg prt, SIR2 fam [Af]	64.2
TM1343	pyrimidine dimer DNA glycosylase (uveA), APM [Mlu]	62.5	TM1485	ribosomal prt L6 (rplF) [Bst]	72.2	TM1655	response reg DrrA (drrA) [Tm]	100
TM0382	repair endonuclease, put [Mj]	69.7	TM0457	ribosomal prt L7/L12 (rplL) [Tm]	100	TM0399	response reg (Ae)	65.9
TM1736	replicative DNA helicase (dnaB) [Bs]	70.3				TM1360	response reg [Bs]	65.8
TM0173	reverse gyrase (rgy) [Pfu]	63.1				TM0126	response reg [Bs]	60.1
TM0915	ribonuclease HII (mhB) [Hi]	68.3				TM0186	response reg [SPCC]	60.5
						TM0842	response reg [SPCC]	56.7
						TM0468	response reg [Tm]	69.2
						TM0143	response regulator/GGDEF domain [SPCC]	53.0

The inevitable youthfulness of known high-redshift radio galaxies

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Some galaxies are very luminous in the radio part of the spectrum. These 'radio galaxies' have extensive (hundreds of kiloparsecs) lobes of emission powered by plasma jets originating at a central black hole¹. Some radio galaxies can be seen at very high redshifts², where in principle they can serve as probes of the early evolution of the Universe. Here we show that, for any model of radio-galaxy evolution in which the luminosity decreases with time after an initial rapid increase (that is, essentially all reasonable models³), all observable high-redshift radio galaxies must be seen when the lobes are less than 10^7 years old. This means that high-redshift radio galaxies can be used as a high-time-resolution probe of evolution in the early Universe. Moreover, this result explains many observed trends of radio-galaxy properties with redshift^{4–9}, without needing to invoke explanations based on cosmology¹⁰ or strong evolution of the surrounding intergalactic medium with cosmic time⁶, thereby avoiding conflict with current theories of structure formation¹¹.

The luminosity P of the expanding lobes of a classical double radio galaxy with age t depends on the bulk kinetic power delivered

by the jets, Q . It will also be influenced by its environment, which we parametrize at radius r as $n(r) \propto r^{-\beta}$, where $n(r)$ is the number density of gas particles. A good empirical fit to radial density profiles^{12,13} is obtained using $\beta = 1.5$. It is also a reasonable approximation to the so-called 'universal' density profile which emerges from N -body simulations of structure formation¹⁴. We normalize the density profile by consideration of (1) detections of thermal X-ray haloes¹⁵, (2) weak and strong gravitational lensing^{16,17}, (3) galaxy counting¹⁸, and (4) studies of radio depolarization⁸, obtaining a characteristic number density n_{100} at radius 100 kpc of $n_{100} = 2 \times 10^3 \text{ m}^{-3}$. Falle¹⁹ found that the length of a radio galaxy, D , grows as:

$$D \propto \left(\frac{t^3 Q}{n_{100}} \right)^{\frac{1}{5-\beta}} \quad (1)$$

A simple model for the evolution of the luminosity of a radio source assumes that Q remains constant as t increases. Combining equation (1) with a minimum-energy estimate of the energy stored in the lobes leads to:

$$P \propto Q^{(26-7\beta)/(4(5-\beta))} n_{100}^{9/[4(5-\beta)]} t^{(8-7\beta)/(4(5-\beta))} \quad (2)$$

Using $\beta = 1.5$ gives:

$$P \propto Q^{31/28} n_{100}^{9/14} t^{-5/28} \quad (3)$$

Equations (1) and (2) can be used to produce evolutionary tracks of P versus D for radio sources of given Q and n_{100} (Fig. 1a). A model²⁰ which incorporates synchrotron cooling, adiabatic expansion losses and inverse Compton scattering off the cosmic microwave background modifies these tracks slightly (Fig. 1a) and, at large D , introduces downward curvature.

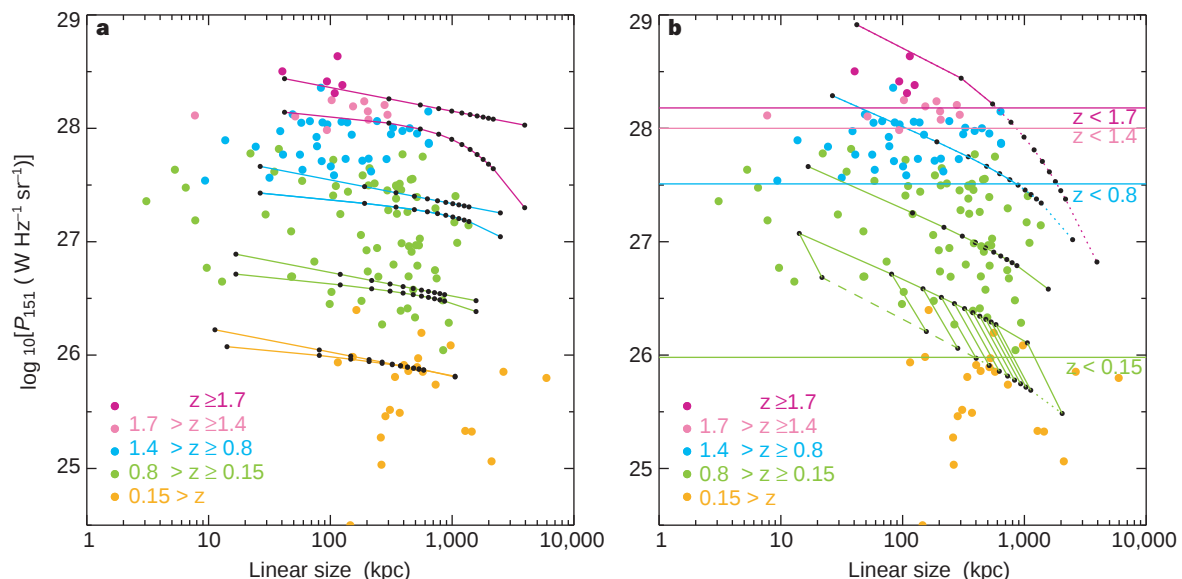


Figure 1 Luminosity of radio sources at a rest-frame frequency of 151 MHz versus linear size. The tracks were generated assuming linear size growth according to the prescription of ref. 19. Small dots lie on all tracks to indicate the (rest-frame) ages 0.1, 1, 10, 20 ... 100, 200 Myr. The jet-powers and redshifts are given by: (upper to lower) $Q = 5 \times 10^{39} \text{ W}$ at $z = 2$, $Q = 1 \times 10^{39} \text{ W}$ at $z = 0.8$, $Q = 2 \times 10^{38} \text{ W}$ at $z = 0.5$ and $Q = 5 \times 10^{37} \text{ W}$ at $z = 0.15$. The large coloured circles show the rest-frame luminosities at 151 MHz of members of the 3C sample³² against their projected linear sizes. **a**, The luminosity development of an individual source is described by equation (3) (straight tracks) and by the model of ref. 20 (curved tracks). **b**, These tracks were generated assuming the model for the luminosity evolution wherein the hotspot acts as a governor of the energy distribution to the radio lobes, developed in ref. 7. The solid lines of the tracks become dotted when

the luminosity and redshift of that source are such that they fall below the survey flux-limit of 12 Jy. The uppermost horizontal line indicates that if a source of that luminosity is to be above the flux-limit at 151 MHz of the 3C sample (12 Jy), then it must be at a redshift closer than 1.7; the corresponding lines at lower luminosities are for the lower redshifts 1.4, 0.8 and 0.15, respectively. The assumed environmental parameters are $n_{100} = 2 \times 10^3 \text{ m}^{-3}$ and $\beta = 1.5$ outside a core radius a_0 of 10 kpc, and $\beta = 1$ inside; the normalizing constant²⁰ c_1 for equation (1) is 3.5. $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, $\Omega_M = 1$ and $\Omega_\Lambda = 0$. The dashed line in **b** indicates how the lower track luminosity reduces by less than about half an order of magnitude if the ambient density (hence n_{100}) becomes an order of magnitude lower.

Our study of the properties and dependencies of complete samples⁷ of radio sources led us to a model which includes the role of the hotspots (the compact, high surface-brightness regions at the end of jets) in governing the energy distribution of the particles injected into the lobes and in promoting increasing expansion losses throughout a radio galaxy's life. In this model the P - D tracks are, for a given environment, steeper (see Fig. 1b) than for the models in Fig. 1a. After a very rapid initial increase, the luminosity of the radio lobes will decrease as the source grows, for $\beta \gtrsim 0.3$ in the case of the last model (Fig. 1b), and for $\beta > 8/7$ in the case of the first and second models (described by equation (2) and ref. 20, respectively) (Fig. 1a). With $\beta \approx 1.5$, radio luminosity inevitably declines with t .

Application of a low-frequency flux limit will inevitably lead to more distant sources selected by a survey being more radio luminous (this is called the Malmquist bias). From equation (2), this means that the most distant objects in a survey are drawn from a higher- Q population. As the dependence of P on Q is stronger than on n_{100} , and as the dynamic range in Q is larger than that of n_{100} , jet-power Q is the dominant factor, though density n_{100} may be an important source of subtle biases. If, as we have argued, the luminosities of radio sources decrease as they age, more distant sources fall through the flux-limit sooner than low-redshift sources. As our light-cone intercepts any radio source at a random point in its life-time⁷, only the high-redshift radio sources which are intercepted by our light-cone when they are young and luminous can be selected by these surveys. Any model involving declining luminosity evolution will give a 'youth-redshift degeneracy'. Although it has been demonstrated that the low-frequency surveys²¹ are unlikely to be much affected by surface-brightness dimming, any effect will be in the sense to make radio galaxies at high redshift even harder to detect.

The youth-redshift degeneracy is highly relevant to our understanding of the alignment effect⁴, the optical and infrared light which is aligned along radio-jet axes. Where this effect is caused by star formation, it will be more easily triggered close into the host galaxy or within the product of a recent merger (assuming this is the jet-triggering event²²) than at distances further out sampled by the

head of an expanding radio source later in its lifetime. Where the alignment effect is caused by dust-scattered quasar light, the certain youthfulness of distant radio galaxies alleviates the near-discrepancy²³ between radio-source ages and the timescale for which dust grains can survive in the presence of shocks caused by the advancing radio jets. The 'youth-redshift degeneracy' is consistent with the finding that the smallest sources in a sample of $z \approx 1$ radio galaxies (all with very similar luminosities) are those which are most aligned with optical emission²⁴. Indeed, Best *et al.*²⁴ remarked that the sequence of changing optical aligned structure with increasing radio size could be naturally interpreted by comparing it with different phases of the interaction of the radio jets with the interstellar and intergalactic medium as the radio sources age.

Barthel and Miley⁶ had suggested that higher-redshift environments are denser and more inhomogeneous than at low redshift because they found increased distortion in the structures of their high- z sample of steep-spectrum quasars compared with their low- z sample. Sources which are younger may have the passage of their jets considerably more disrupted where there is a higher density and greater inhomogeneity in the ambient post-recent-merger environment. A general trend of denser intergalactic environments at high z cannot be inferred from their result.

The mild linear size evolution observed in low-frequency selected samples of classical double radio sources⁵⁻⁷ arises because the high- z sources are younger, hence tend to be shorter. It is the positive dependence on jet-power of the rate at which the lobe-lengths grow (equation (1)) which contributes to the linear size evolution being as mild as it is⁷. Because we find it unavoidable that objects found at high redshift will be younger than those at low redshift, the use of classical double radio sources as 'standardizable' rods¹⁰ is unwarranted. Figure 2 illustrates the difficulty of distinguishing between different underlying cosmic geometries when more dramatic influences—such as the youth-redshift degeneracy and variations in source environments—are at work. Garrington and Conway⁸ have found a tendency for depolarization to be higher in sources with a higher P and/or z . Objects with higher P or z which are younger will be in much more recently merged environments, with the conse-

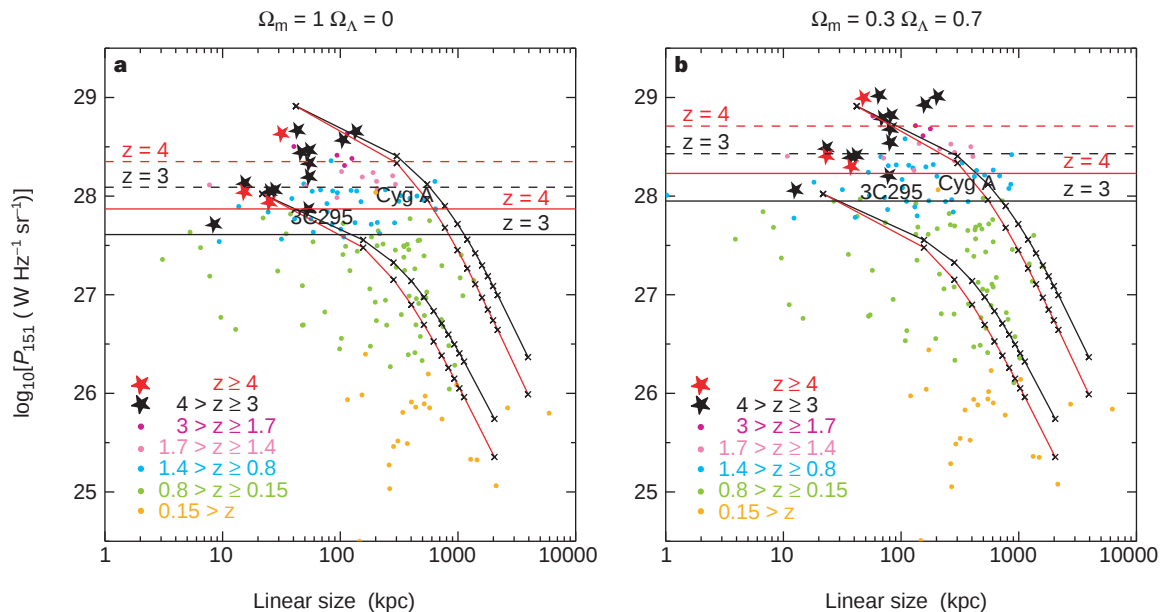


Figure 2 Tracks generated according to the same model as Fig. 1b, with parameters as in Fig. 1a. The coloured circles have the same meaning as in Fig. 1. The black stars represent the known radio galaxies with $3 < z < 4$ and the red stars are those with $z > 4$. The dashed horizontal lines indicate the luminosity a source must have if it is to be above a flux-limit of 3 Jy at $z = 4$ (red) and $z = 3$

(black). The solid horizontal lines are for a flux-limit of 1 Jy. Cygnus A and 3C295 are indicated. The upper pair of tracks have $Q = 5 \times 10^{38}$ W and the lower pair have $Q = 5 \times 10^{36}$ W; the red tracks are for sources at $z = 4$ and the black tracks are for $z = 3$. Higher jet-powers must be invoked if $\Omega_m = 0.3$ and $\Omega_\Lambda = 0.7$ than if $\Omega_m = 1$ and $\Omega_\Lambda = 0$.

quence that inhomogeneities in density or magnetic field will more readily depolarize the synchrotron radiation from the lobes, in addition to being closer in to the centre of the potential well.

Many of the highest- z radio galaxies have gas masses comparable to gas-rich spiral galaxies⁹, and inferred star-formation rates which, in the local Universe, are rivalled only by galaxy-galaxy mergers like Arp 220²⁵. If high- z objects are being viewed during a similar merging of sub-components, the associated star formation could be responsible for a significant fraction of the stellar mass in the remnant galaxy. As we have shown (Fig. 1) that the high- z radio galaxies—those detected by SCUBA—are necessarily young ($\leq 10^7$ yr), and as the whole merger must take a few dynamical crossing times, or 10^8 – 10^9 yr, the implication is that the event which triggered the jet-producing central engine is synchronized with massive star formation in a gas-rich system, perhaps as material streams towards the minimum of the gravitational potential well of the merging system. The youth–redshift degeneracy may explain why few lower- z radio galaxies show similarly large (rest-frame) far-infrared luminosities compared to the high- z population: they are being observed significantly longer after the jet-triggering event.

The dramatic decrease in the co-moving space density²⁶ of radio-galaxies from $z \approx 2$ to the local Universe is most likely to be explained by the probability of suitable jet-triggering events like galaxy–galaxy mergers²² being much lower now than at earlier cosmic epochs. Cygnus A is a local example demonstrating that this probability is not yet zero. Because efficacious interactions and mergers require slow relative motions²⁷, rich galaxy groups are excellent sites for jet-triggering events by virtue of their high galaxy number density but low velocity dispersion before virialization. This is evinced by studies of the dynamics of rich groups containing luminous quasars²⁸, which find anomalously low velocity dispersions for systems of high galaxy number density. But if systems, whose galaxy number densities are sufficiently high that galaxy encounters are frequent, have formed into large virialized clusters $z \approx 0$, then the galaxies' encounter velocities will be prohibitively high. This explains why radio-source environments may be seen to get richer between $z \approx 0$ and $z \approx 0.5$ (refs 18, 29). The space density evolution of radio sources may therefore be understood in the context of current structure-formation theories¹¹. Only in systems where the merger of sub-groups is still continuing are jets likely to be triggered in the local Universe: Cygnus A was recently shown to be embedded in a continuing merger of two sub-clusters^{30,31}.

Figure 2 shows the location on the P – D plane of the most extreme redshift ($z > 3$) radio galaxies known. Figure 2a (where we take $\Omega_M = 1$ and $\Omega_\Lambda = 0$) (Ω_M and Ω_Λ are the dimensionless density parameters for matter and the cosmological constant, respectively) suggests that not only are the extreme-redshift sources younger than the most distant objects in the 3C sample³², but also that they have similar jet-powers. Figure 2b ($\Omega_M = 0.3$, $\Omega_\Lambda = 0.7$) requires that objects with higher jet-powers ($\sim 10^{40}$ W) exist beyond those detected in the $z < 2$ Universe sampled by 3C. The extreme values of Q required in even the most conservative cosmological model constrains their jet-producing active galactic nuclei to be only those with the most massive black holes. A jet-power of 10^{40} W corresponds to a 100%-efficient Eddington-rate process for a black hole of 10^9 solar masses. Unless one is prepared to postulate significantly larger black-hole masses, it seems difficult to avoid the conclusion that by focusing on these objects, we are selecting objects from the extreme end of the distribution function of black-hole masses. Interpretation of the properties of distant radio galaxies must account for this effect alongside the youth–redshift degeneracy.

We note that the term 'cosmological evolution' should not be used to describe trends with redshift where they arise because of well-understood physical mechanisms. The youth–redshift degen-

eracy is one example where trends with source age have become confused with changes with cosmic epoch. However, the youth–redshift degeneracy brings two important benefits: first, as extreme-redshift radio galaxies are young, all with similar Q , they deliver the fine time-resolution required for the solution of problems which it may be difficult to study with objects like optically selected quasars, whose ages are indeterminate: examination of the environments of distant radio galaxies provides a snapshot of the host galaxy evolutionary status just after the jet-triggering event. Second, at redshift ≈ 4 we observe radio galaxies ~ 1 Gyr after the Big Bang and in environments which saw a jet-triggering event a time-step no greater than 10^7 years before that. This time-step is over an order of magnitude smaller than the dynamical crossing time of a massive galaxy, and two orders of magnitude smaller than the age of the Universe at the epochs probed, giving the fine time-resolution essential to any study of triggering (and hence merging) rates at early cosmic epochs.

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Zero-point entropy in 'spin ice'

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Common water ice (ice I_h) is an unusual solid—the oxygen atoms form a periodic structure but the hydrogen atoms are highly disordered due to there being two inequivalent O–H bond lengths¹. Pauling showed that the presence of these two bond lengths leads to a macroscopic degeneracy of possible ground states^{2,3}, such that the system has finite entropy as the temperature tends towards zero. The dynamics associated with this degeneracy are experimentally inaccessible, however, as ice melts and the hydrogen dynamics cannot be studied independently of oxygen motion⁴. An analogous system⁵ in which this degeneracy can be studied is a magnet with the pyrochlore structure—termed 'spin ice'—where spin orientation plays a similar role to that of the hydrogen position in ice I_h . Here we present specific-heat data for one such system, $Dy_2Ti_2O_7$, from which we infer a total spin entropy of $0.67R\ln 2$. This is similar to the value, $0.71R\ln 2$, determined for ice I_h , so confirming the validity of the correspondence. We also find, through application of a magnetic field, behaviour not accessible in water ice—restoration of much of the ground-state entropy and new transitions involving transverse spin degrees of freedom.

The problem of hydrogen-ordering in ice is perhaps the first example of geometrical frustration, by now a familiar concept in magnetism⁶, where suppression of long-range magnetic order arises from the incompatibility of local and global symmetries. The simplest such case is realized for uniaxial spins interacting antiferromagnetically on a two-dimensional triangular lattice. This system does not order magnetically at any finite temperature and has, theoretically, a finite ground-state entropy⁷. In ice I_h , geometrical frustration arises from the incompatibility of the local bonding distances with long-range oxygen order. The oxygens form a periodic lattice isomorphic to the centre of the tetrahedra in the pyrochlore structure shown in Fig. 1. The O–O bond length is 2.75 Å, which is greater than twice the covalent O–H distance of 1.00 Å. In order to reconcile the crystal structure of ice I_h with the known bond lengths, Bernal and Fowler¹ formulated the so-called ice rules, which stipulate that two of the four hydrogen ions surrounding each oxygen ion sit closer to the oxygen ion than the other two. For every tetrahedron, there are six ways of achieving this lowest energy state and because the tetrahedra are corner-sharing, the associated degeneracy can not be lifted by long-range order. Pauling showed² that this leads to residual "macroscopic" ground-state entropy per H of $(1/2)R\ln(3/2) = 1.68 \text{ J mol}^{-1} \text{ K}^{-1}$. This idea resolved a discrepancy between the spectroscopically determined entropy at 298 K and that determined by direct calorimetric measurements³. Although the H-disorder can be represented as ground-state entropy, there is no clear violation of the third law of thermodynamics because the energetic barriers to establishing long-range order are of the order $\sim 1 \text{ eV}$, which makes relaxation through tunnelling into a lower entropy state an extremely slow process below ice's freezing temperature.

The analogous magnetic situation is one where the two H-positions (close to, and far from, an O atom) are represented by the two states of spin-1/2 atomic moments, which reside on a lattice of corner-shared tetrahedra, as in the pyrochlore lattice (Fig. 1). Additional requirements for the analogy are that the spins lie along an axis (Ising-type) joining the tetrahedron vertex with its centre,

and that they interact ferromagnetically. Harris *et al.*⁵ showed that in this case, the lowest energy state for each tetrahedron is one where two spins point outwards and two point inwards, a state which has the same degeneracy as ice I_h . This situation is realized in the rare-earth (RE) titanate pyrochlores $RE_2Ti_2O_7$ where RE is Dy^{3+} or Ho^{3+} . (Dy^{3+} has effective spin $\tilde{S} = 1/2$ and an effective g-factor governing Zeeman splitting of the ground-state doublet $g_{\text{eff}} \approx 9$ along the Ising axis. The next highest excited state is expected to be around 200 K.) The Ising nature of Dy and Ho is to be contrasted with the more common Heisenberg (or x - y) types of spin, where vector character is manifested in a continuous range of possible spin orientations and where, consequently, ice rules do not apply. Systems where such spins are situated in structures which are (like the pyrochlore lattice) both underconstrained in the Maxwellian sense⁸ and triangle-based also have large ground-state degeneracy, and are said to be geometrically frustrated^{6,9}. The analogy of the Ising-spin system with ice I_h has led Harris *et al.* to call this system "spin ice"⁵—its advantage over ice I_h is that the structure is stable at temperatures greater than the average interaction strength, allowing the study of the development of the degenerate ground state.

The $Dy_2Ti_2O_7$ sample used in the present measurements of heat capacity (C) as a function of temperature (T) was a ceramic powder prepared by standard solid-state synthesis techniques. The starting materials— Dy_2O_3 (99.99%) powder and TiO_2 (99.995%) powder—were mixed in stoichiometric proportions and heated in air at 1,200 °C for 4 days. For $C(T)$ measurements, the resulting powder was cold-sintered into a hard disk with Ag powder to facilitate thermal equilibration. The mass of $Dy_2Ti_2O_7$ in the disk was determined by measuring d.c.-susceptibility, χ_{dc} , above 2 K, and compared to a sample of precisely known mass. The Ag contribution to $C(T)$ was measured separately and subtracted from all data; at 1 K it represented only 0.4% of the total heat capacity. A standard semiadiabatic heat pulse technique was used with an equilibration time window of 15 s. Below 0.5 K in zero field and at lower T in finite fields, the internal relaxation time merged with the external relaxation time mostly as a result of a very small $C(T)$, and thus $C(T)$ was not well defined below $T \approx 0.2 \text{ K}$.

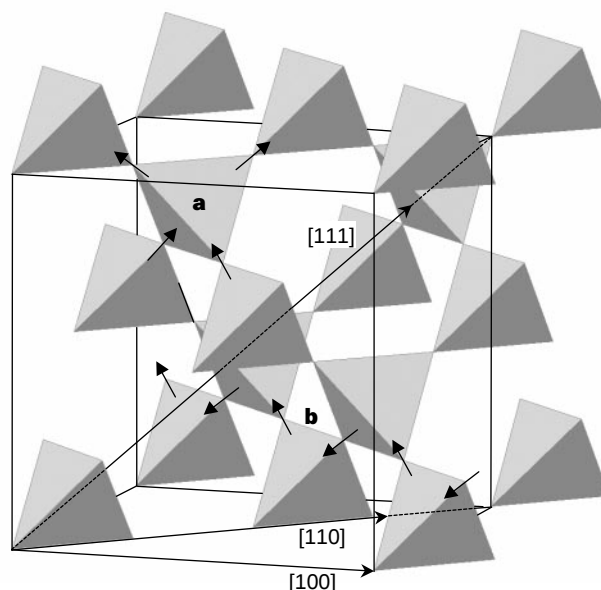


Figure 1 A section of the pyrochlore lattice, with the unit cell shown. Also shown are the principal crystal axes and two different spin configurations, discussed in the text, for Dy spins which lie on the vertices of the tetrahedra. Panel **a** shows the ground-state configuration of ferromagnetically interacting Ising spins on a tetrahedron. Panel **b** shows part of the subfamily of spins which do not couple to a field along [110].

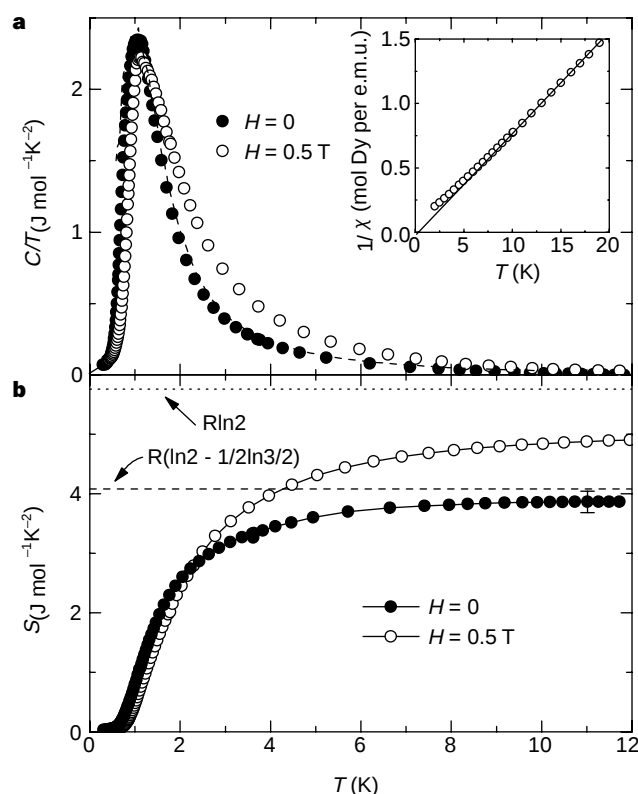


Figure 2 Specific heat and entropy of the spin-ice compound $\text{Dy}_2\text{Ti}_2\text{O}_7$ showing agreement with Pauling's prediction for the entropy of water ice I_h , $R(\ln 2 - (1/2)\ln(3/2))$. **a**, Specific heat divided by temperature of $\text{Dy}_2\text{Ti}_2\text{O}_7$ in $H = 0$ and 0.5 T. The dashed line is a Monte Carlo simulation of the zero-field $C(T)/T$, as discussed in the text. **b**, Entropy of $\text{Dy}_2\text{Ti}_2\text{O}_7$ found by integrating C/T from 0.2 to 14 K. The value of $R(\ln 2 - (1/2)\ln(3/2))$ is that found for ice I_h , and $R \ln 2$ is the full spin entropy. Inset, susceptibility (M/H) of $\text{Dy}_2\text{Ti}_2\text{O}_7$ in a field of 0.02 T.

In Fig. 2a inset we show $\chi_{\text{dc}}(T)$ from 2 to 20 K, illustrating the small ferromagnetic, FM, intercept, corresponding to a Weiss constant $\theta_w \approx 0.5$ K, where $1/\chi = \text{const.}/(T - \theta_w)$. The $C(T)/T$ data, which extend down to lower temperatures (Fig. 2a), show a much broader peak than usually seen for an antiferromagnetic, AF, transition. The lack of a clear ordering feature in $C(T)$ is consistent with a picture where the spins 'freeze' in a random configuration as a result of geometrical frustration. The absence of magnetic order in a system with no structural disorder is by itself unusual. The first reported example of such a system is another pyrochlore compound, $\text{Y}_2\text{Mo}_2\text{O}_7$, where despite the absence of any measured structural disorder, long-range magnetic order is not observed¹⁰—instead, spin glass freezing among Heisenberg-like Mo^{4+} ions sets in at $T \approx 0.3\theta_w \approx 15$ K. But existing susceptibility measurements¹¹ on $\text{Dy}_2\text{Ti}_2\text{O}_7$ do not show the sharp cusp expected for a spin glass, but rather a broad feature peaked at $T \approx 0.7$ K, indicating a different type of frozen spin state for this Ising-type spin system.

The most surprising aspect of our data, however, is found when integrating $C(T)/T$ from 0.2 to 12 K to obtain the total spin entropy (Fig. 2b). This temperature range incorporates all appreciable observed contributions to $C(T)/T$. We obtain $\Delta S(0.2, 12) = (0.67 \pm 0.04)R \ln 2$, that is, a shortfall of $\sim 1/3$ of the total spin entropy. It has been previously noted, based on measurements of $C(T)$ only up to 1.5 K and a numerical extrapolation to higher temperatures, that the peak height is consistent with reduced entropy¹¹; but it was suggested that the extrapolation was too simple, and that the missing entropy would be found for $T > 1.5$ K. We see no evidence for missing entropy for $T > 1.5$ K

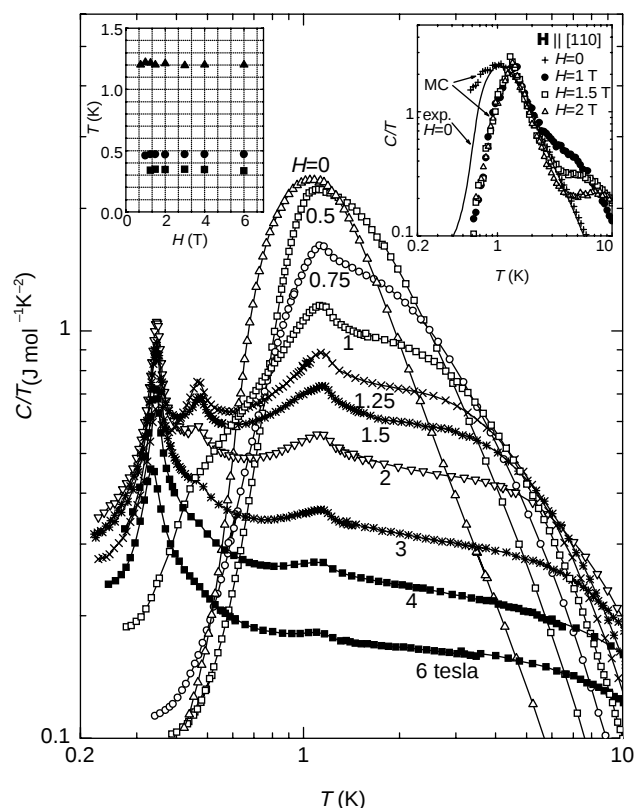


Figure 3 Specific heat versus temperature for various values of applied field. The broad $H = 0$ feature is suppressed on increasing H and replaced by three sharp features at $0.34, 0.47$ and 1.12 K. The left inset shows the constancy of these transition temperatures with field; the right inset shows the results of finite-field Monte Carlo (MC) simulations of C/T .

and, although it is possible that additional entropy is developed below 0.2 K, we think it unlikely for the following reasons. First, $C(T)/T$ drops by almost two orders of magnitude from 1 to 0.5 K indicating near-complete spin freezing, and second, there is no structural reason to assume a bimodal distribution of entropy-loss processes, for example, due to two different exchange interactions. In addition, our Monte Carlo simulation reproduces the observed $C(T)/T$ peak height and shape (Fig. 2a). (The Monte Carlo simulation was performed on a sample of size $8 \times 8 \times 8$ tetrahedra ($2,048$ spins) and $\sim 10^4$ Monte Carlo steps per spin. The spin–spin interaction was assumed to be purely dipole–dipole but with a g -factor reduced by 25% from the $J = 15/2$ Landé value. This is most likely the result of the compensating effect of a small admixture of superexchange interaction. Justification for this, and further details, will be given elsewhere (A.P.R. *et al.*, manuscript in preparation).

The comparison of the measured entropy with the prediction of Pauling for ice I_h , $R(\ln 2 - (1/2)\ln(3/2))$, is shown in Fig. 2b. To test the idea that there exists a contribution to ground-state entropy from a different energetically unfavoured state, we applied a small magnetic field, H , to reduce the energy barriers for spin reorientation. As shown in Fig 2a and b, an applied field of 0.5 T results not only in a shift of $C(T)/T$ to higher temperatures, but also in an increase of the integrated entropy, $\Delta S(0.2, 12)$, from $0.67R \ln 2$ to $0.85R \ln 2$. The increase of temperature where $C(T)/T$ is appreciable is expected, because Zeeman splitting increases with field. The increase of total ΔS , however, underscores the existence of additional entropy beyond that contained in the $H = 0$ peak. The

energetic barriers to spin reorientation, which are presumably due to geometrical frustration, are effectively lowered in finite field. The analogous experiment to recover missing entropy by application of pressure has not been performed in ice I_h , to our knowledge, although KOH-doping induces a transition which seems to restore nearly all of the missing entropy¹².

For applied fields larger than 1 T, additional features develop in $C(T)/T$ in the form of three sharp peaks at $T = 0.34, 0.47$ and 1.12 K (Fig. 3). These peaks are unusual in two respects. First, they represent only a few per cent of the total spin entropy—this is most likely to be due to the polycrystalline nature of the sample, as discussed below. Second, the peak positions do not depend on the magnitude of the applied field. This field-independence is shown in Fig. 3, left inset. We note that, independent of the details of possible spin-ordering models, these peaks can not be due to longitudinal spin fluctuations—they are still observable at $H = 6$ T where the Zeeman energy for longitudinal spins is ~ 55 K, that is, a Boltzmann factor $g\mu_B\hbar H/k_B T$ of 157 for the lowest-temperature peak.

The occurrence of sharp peaks in thermodynamic quantities in finite field when there are none in zero field is rare, and to our knowledge has been seen previously only in the frustrated magnet $Gd_3Ga_2O_{12}$ (refs 13, 14) and low-dimensional spin systems¹⁵. In contrast to both of these examples, the peak temperatures in $Dy_2Ti_2O_7$ are field-insensitive. Harris *et al.* have recently reported¹⁶ Monte Carlo simulations of $C(T)$ for the pyrochlore ferromagnet. These workers found, on application of a finite field $H||[100]$, a sharp spike in $C(T)$; the temperature corresponding to the peak of this spike also varied with applied field, indicating a different type of transition to that which we observe in $Dy_2Ti_2O_7$.

We propose that the $Dy_2Ti_2O_7$ peaks are due to correlated motion among spins which do not couple to the magnetic field, a situation realized for $H||[100]$ where half the spins have their Ising axis oriented perpendicular to H . In this case, for $g\mu|H| \gg k_B\theta_w$, the disorder of the ice state will be suppressed, leading to ordering on the sublattice of field-decoupled spins, which form chains as shown in Fig. 1. This model is supported by our own Monte Carlo calculations which show that for $H||[100]$, a sharp field-independent peak develops at a temperature corresponding to that of the upper peak in the $Dy_2Ti_2O_7$ data (Fig. 3, right inset). The observed lower- T transitions are possibly long-wavelength reordering processes which are not reproduced in Monte Carlo simulations owing to the small sample size in such simulations. Finally, we note that the peak heights in our orientationally averaged powder sample are significantly less than expected from ordering among half the spins. This suggests that there will need to be tight constraints on the sample orientation in future experiments on single crystals. □

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A thermoacoustic Stirling heat engine

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Electrical and mechanical power, together with other forms of useful work, are generated worldwide at a rate of about 10^{12} watts, mostly using heat engines. The efficiency of such engines is limited by the laws of thermodynamics and by practical considerations such as the cost of building and operating them. Engines with high efficiency help to conserve fossil fuels and other natural resources, reducing global-warming emissions and pollutants. In practice, the highest efficiencies are obtained only in the most expensive, sophisticated engines, such as the turbines in central utility electrical plants. Here we demonstrate an inexpensive thermoacoustic engine that employs the inherently efficient Stirling cycle¹. The design is based on a simple acoustic apparatus with no moving parts. Our first small laboratory prototype, constructed using inexpensive hardware (steel pipes), achieves an efficiency of 0.30, which exceeds the values of 0.10–0.25 attained in other heat engines^{5,6} with no moving parts. Moreover, the efficiency of our prototype is comparable to that of the common internal combustion engine² (0.25–0.40) and piston-driven Stirling engines^{3,4} (0.20–0.38).

We plan to acoustically couple our thermoacoustic Stirling engines to pulse tube refrigerators⁷, to provide efficient and maintenance-free combustion-powered cryogenic refrigeration having no moving parts, in order to liquefy natural gas⁸. This would enable economic recovery of ‘associated gas’, a by-product of oil production, which is currently burned (‘flared’) in vast quantities at remote oil fields worldwide, wasting fossil fuel and contributing to global warming. We expect our engine to find many additional uses throughout the global power-production environment, ranging from the separation of air into nitrogen and oxygen to the generation of electricity.

Ceperley^{9,10} made the initial step towards this new engine, when he realized that gas in an acoustic travelling wave propagating through a regenerative heat exchanger (regenerator) undergoes a thermodynamic cycle similar to the ideal Stirling cycle; this is because the oscillations of pressure (p_1) and volumetric velocity (U_1) are temporally in phase in a travelling wave. We use the conventional anticlockwise phasor notation¹¹, so that time-dependent variables are expressed as

$$\xi(t) = \xi_m + \text{Re}[\xi_1 e^{i\omega t}] \quad (1)$$

with the mean value ξ_m real, and with ξ_1 complex, to account for both the magnitude and phase of the oscillation at angular frequency ω . The net work performed in the cycle manifests itself as an increase in the time-averaged acoustic power $\dot{W} = \text{Re}[p_1 \tilde{U}_1]/2$ of the wave as it passes through the regenerator. (The tilde denotes complex conjugate.) Although Ceperley’s experiments never achieved power gain, he provided a key insight¹² for constructing a useful thermoacoustic Stirling engine. If a pure travelling wave were used, the ratio of acoustic pressure to volume velocity would be

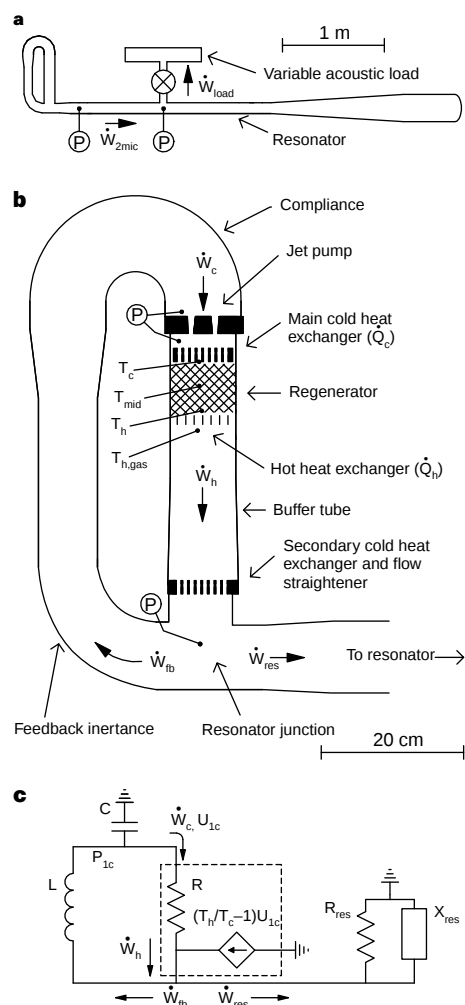


Figure 1 The thermoacoustic Stirling heat engine, constructed from steel pipe. **a**, Scale drawing of the engine. The variable acoustic load consists of an adjustable valve leading to a 2.2-l tank²⁰. The valve setting controls the acoustic power flowing into the load $\dot{W}_{load}$, and hence T_h . Piezoresistive pressure sensors 'P' allow measurement of²⁰ the acoustic power flowing past their midpoint, $\dot{W}_{2mic}$. **b**, Details of the torus-shaped section. The regenerator is a stack of 120-mesh stainless-steel screen whose hydraulic radius is $\sim 42 \mu\text{m}$. This is smaller than the average thermal penetration depth of the helium ($300 \mu\text{m}$), ensuring the good thermal contact between the helium gas and the screen necessary for thermodynamic reversibility. The two cold heat exchangers are of shell-and-tube construction, with the oscillating helium in the tubes and flowing cooling water in the shell. The hot heat exchanger is electrically heated Ni-Cr ribbon wound in a zig-zag fashion on an alumina frame. The buffer tube provides thermal insulation between the hot heat exchanger and ambient temperature while transmitting the acoustic power out of the hot zone. Its internal surface is slightly tapered and polished to reduce boundary-layer streaming, which would otherwise convect heat from the hot heat exchanger¹⁶. Thermal insulation around the hot components is not shown. The two tapered channels of the jet pump are 51 mm long and 34 mm high, and their short, tapered dimensions are adjustable, typically set in the range 1–2 mm. The lower edges of the channels are rounded with a 0.8-mm radius, to reduce K_{in} to nearly zero (see text). The taper increases the cross-sectional area at the upper end, thereby decreasing the velocity there and preventing the upper end from developing an equal and opposite $\Delta\bar{p}_p$ (see text). The temperature of the helium is measured at locations labelled 'T' using 1.6-mm-diameter type-K thermocouples. **c**, Impedance model of the engine. The dashed lines enclose the regenerator; its volumetric-velocity source $(T_h/T_c - 1)U_{1c}$ is due to the temperature gradient in the regenerator and causes the acoustic power gain. The resonator and variable acoustic load are modelled as a parallel combination of R_{res} with a reactive impedance X_{res} .

fixed at $^{13} p_1/U_1 = \rho_m c/A$ where ρ_m is the mean gas density, c is the speed of sound, and A is the cross-sectional area of the waveguide containing the wave. For realistic gases, U_1 would then be large enough to cause significant viscous losses, which would overwhelm any power gain. Hence, it is crucial to make $p_1/U_1 \gg \rho_m c/A$ within the regenerator, while maintaining travelling-wave phasing, to achieve a large acoustic power gain with a minimum of viscous loss.

Scale drawings of our engine, which achieved such an unusual condition, are shown in Fig. 1 a and b. Overall, it comprises a 1/4-wavelength acoustic resonator filled with helium at a pressure of 30 bar. The torus-shaped section contains the heat exchangers, regenerator, inductance, L (due to the inertia of the helium) and compliance, C (due to the compressibility of the helium) necessary to force the helium to execute the Stirling cycle. The inlet to the torus is near a pressure antinode (velocity node) which helps achieve $p_1/U_1 \gg \rho_m c/A$ within the regenerator. Applying heat to the hot heat exchanger creates an intense acoustic wave at the resonance frequency, 80 Hz.

Figure 1c shows a simplified acoustic impedance diagram of the engine, which captures the essential dynamics of the engine operation but removes the complicated spatial dependence of the acoustic wave. Each acoustic component is analogous to a similar electrical component, with p_1 analogous to a.c. voltage and U_1 analogous to electrical current⁹. Following Ceperley⁹, the regenerator and adjacent heat exchangers are modelled as a resistance R and a volumetric-velocity source $(T_h/T_c - 1)U_{1c}$, where U_{1c} is the volumetric velocity into the cold end of the regenerator, and T_h and T_c are the temperatures of the hot and cold faces of the regenerator, respectively.

The study of this simple model provides basic insights into the operation of the engine. Solving for U_{1c} , we find:

$$U_{1c} = \frac{\omega^2 LC}{R} \frac{p_{1c}}{1 + i\omega L/R} \quad (2)$$

If the impedance ωL of the feedback inductance L is small compared with the resistance R of the regenerator, p_{1c} and U_{1c} are nearly in phase, corresponding to the travelling-wave phasing necessary to drive the helium through the Stirling cycle, yet the ratio $|p_{1c}/U_{1c}|$ is no longer determined by $\rho_m c/A$. Here, p_{1c} is the oscillating pressure at the cold end of the regenerator. In our experiments, the physical dimensions of the inductance, compliance and regenerator are chosen so that $|p_{1c}/U_{1c}| \approx 30\rho_m c/A$ so that viscous dissipation in the regenerator is reduced to an acceptable level. As the simple model in Fig. 1c shows, the LRC acoustic network of Fig. 1b provides both the travelling-wave phasing necessary to produce the Stirling cycle and the large value of $|p_{1c}/U_{1c}|$ necessary to avoid large viscous losses.

As this wave propagates through the regenerator, it is amplified by the imposed temperature gradient, resulting in $\dot{W}_h > \dot{W}_c$, where $\dot{W}_c$ and $\dot{W}_h$ are the acoustic powers flowing into the cold end and out of the hot end of the regenerator, respectively. To maintain the acoustic oscillation, part of $\dot{W}_h$ is fed back through the inductance to the cold end of the regenerator, $\dot{W}_{fb}$. (Due to dissipation in the inductance and compliance, $\dot{W}_{fb} > \dot{W}_c$). The remainder of the power, $\dot{W}_{res}$, is available to maintain the wave throughout the resonator and perform useful work.

In this process, heat $\dot{Q}_h$ is absorbed from the hot heat exchanger and $\dot{Q}_c$ is rejected at the cold heat exchanger. But, in our initial measurements, the $\dot{Q}_h$ needed to sustain the oscillation was far greater than we estimated using a detailed computer model¹⁴ of the system. Also, the temperature profile in the regenerator was far from the estimated linear profile. Both of these effects may be understood by considering the second-order, time-averaged mass flux circulating down through the regenerator and up through the inductance, an effect that was not anticipated by Ceperley. Gedeon¹⁵ has discussed how a mass flux $\bar{M}_2$ can exist in Stirling systems which contain such a closed-loop path. Gedeon argues that $\bar{M}_2$ is given by:

$$\bar{M}_2 = \frac{1}{2} \text{Re}[\rho_1 \bar{U}_1] + \rho_m U_{2,0} \quad (3)$$

Here, $U_{2,0}$ would be the next term in the expansion of equation (1) for volumetric velocity and represents the second-order, time-averaged volume velocity¹⁶. In acoustic oscillations, $\rho_1 \propto p_1$. Therefore, the first term in equation (3) is non-zero whenever acoustic power flow is non-zero; for example, around the LRC loop of Fig. 1c. This mass flux carries heat away from the hot heat exchanger and deposits it at the secondary cold heat exchanger, creating an unwanted heat leak $\dot{Q}_{\text{leak}} = \dot{M}_2 c_p (T_h - T_c)$ where c_p is the constant-pressure heat capacity of the helium. $\dot{Q}_{\text{leak}}$ can more than double the $\dot{Q}_h$ needed to maintain the acoustic oscillation, seriously degrading the engine's efficiency. The same $\dot{M}_2$ also injects cold gas into the cold end of the regenerator, causing its axial temperature profile to fall below the predicted linear profile.

Equation (3) shows that $\dot{M}_2$ can be forced to zero if a $U_{2,0}$ is imposed such that $\rho_m U_{2,0} = -\text{Re}[\rho_1 \tilde{U}_1]/2$. A time-averaged pressure drop, $\overline{\Delta p_2}$, imposed across the regenerator will drive such a $U_{2,0}$. The $\overline{\Delta p_2}$ required to suppress $\dot{M}_2$ is given by:

$$\overline{\Delta p_2} = \frac{R}{2\rho_m} \text{Re}[\rho_1 \tilde{U}_1]. \quad (4)$$

Such a $\overline{\Delta p_2}$ can be generated using the asymmetry of hydrodynamic end effects¹⁷. When flow of high Reynolds number encounters an abrupt transition from a channel of small cross-sectional area to one of much larger area, the transition is accompanied by jetting and turbulence¹⁷. In this flow, Bernoulli's equation does not hold, and an additional pressure head loss given by $\Delta p_{\text{ml}} = (K_{\text{out}}/2)\rho v^2$ occurs, where v is the velocity of the jet as it leaves. The constant $K_{\text{out}} \approx 1$ is termed the 'minor loss coefficient'. When the flow changes direction, entering the small channel, the minor loss coefficient K_{in}

depends on the geometrical details. If the perimeter of the entrance to the small channel is sufficiently rounded^{17,18}, K_{in} can be as small as 0.04. With $K_{\text{out}} \neq K_{\text{in}}$, a time-averaged pressure drop of

$$\overline{\Delta p_{\text{jp}}} = \frac{\rho_m |U_{\text{jp}}|^2}{8a^2} [K_{\text{out}} - K_{\text{in}}] \quad (5)$$

develops across the transition. Here, U_{jp} is the volume velocity at the transition and a is the area of the small channel. The device labelled 'jet pump' in Fig. 1a utilizes these minor losses by channeling the flow above the cold heat exchanger through two parallel tapered rectangular channels that open abruptly into the larger space just above the exchanger. By adjusting the jet area a , $\overline{\Delta p_{\text{jp}}}$ can be set to the pressure given in equation (4), thereby completely eliminating $\dot{M}_2$ and the large, unwanted $\dot{Q}_{\text{leak}}$.

Figure 2 demonstrates the effectiveness of the jet pump. The lowest curve ('lowest' refers to the curves at $\phi = 120^\circ$) in Fig. 2b shows the time-dependent $\overline{\Delta p_{\text{jp}}}(t)$, displayed as a function of the time phase of the acoustic cycle, with a at its fully open value of 3 cm^2 . When time-averaged, this curve does not yield a significant $\overline{\Delta p_{\text{jp}}}$ so a large $\dot{M}_2$ flows down through the regenerator and buffer tube causing a large $\dot{Q}_{\text{leak}}$. To maintain the oscillation at $|p_1/p_m| = 0.036$, nearly $1,500 \text{ W}$ of $\dot{Q}_h$ is necessary, with more than half of this $\dot{Q}_h$ carried away by $\dot{M}_2$. Also, the temperature at the axial midpoint of the regenerator, T_{mid} , is held significantly below $T_{\text{avg}} = (T_h + T_c)/2$ due to the flux of cold gas entering the cold end of the regenerator. The middle curve in Fig. 2b corresponds to $a = 1.8 \text{ cm}^2$ in Fig. 2a. The resulting $\overline{\Delta p_{\text{jp}}}$ generated by the jet pump enforces $\dot{M}_2 = 0$. $\dot{Q}_{\text{leak}}$ is eliminated, and the $\dot{Q}_h$ needed to maintain $|p_1/p_m| = 0.036$ is at its minimum of 630 W . At this operating point $T_{\text{mid}} = T_{\text{avg}}$, demonstrating a linear temperature profile through the regenerator. The highest curve in Fig. 2b corresponds to $a = 0.8 \text{ cm}^2$ in Fig. 2a. The resulting $\overline{\Delta p_{\text{jp}}}$ exceeds that required by equation (4), so $\dot{M}_2 < 0$, implying that the time-averaged mass flux flows up through the buffer tube and regenerator. Now, T_{mid} is greater than T_{avg} due to the hot gas entering the hot end of the regenerator. These data demonstrate that $\dot{M}_2$ causes a significant and unwanted heat load that can be completely eliminated by $\overline{\Delta p_{\text{jp}}}$. Also, the broad minimum in $\dot{Q}_h$ in Fig. 2a shows that the cancellation of $\dot{M}_2$ is insensitive to small variations in a .

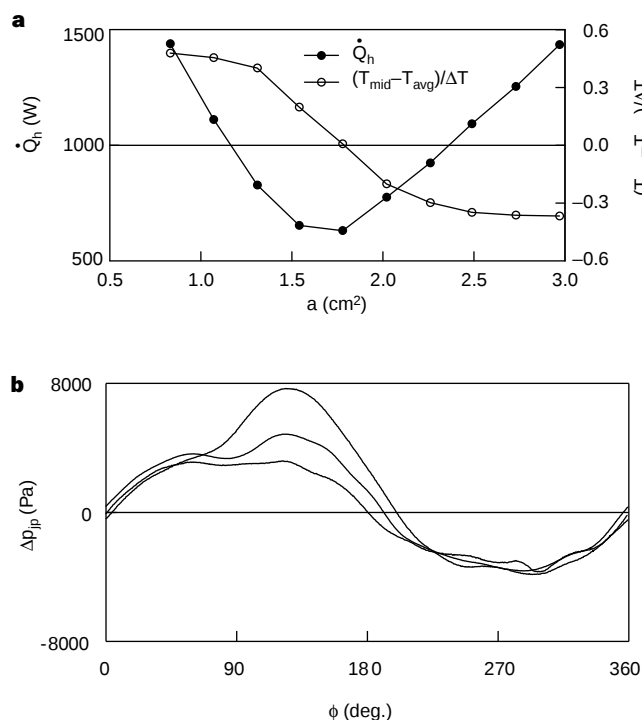


Figure 2 Effectiveness of the jet pump. **a**, Measurements of $\dot{Q}_h$ and $(T_{\text{mid}} - T_{\text{avg}})/\Delta T$ as a function of the jet area a . Here, $\Delta T = T_h - T_c$. As a is varied, $\dot{Q}_h$ is adjusted to maintain $|p_1/p_m| = 0.036$, and hence $\text{Re}[\rho_1 \tilde{U}_1]$ nearly constant within the regenerator. With $a = 1.8 \text{ cm}^2$, $T_{\text{mid}} = T_{\text{avg}}$ indicating that $\dot{M}_2$ is completely suppressed. With $\dot{M}_2 = 0$, $\dot{Q}_{\text{leak}} = 0$ and the heat input needed to maintain the oscillations, $\dot{Q}_h$, is at a minimum. **b**, Measurements of $\overline{\Delta p_{\text{jp}}}(t)$ as a function of the time phase of the acoustic cycle $\phi(t)$. The lowest curve, obtained with $a = 3.0 \text{ cm}^2$, has $\overline{\Delta p_{\text{jp}}} = 120 \text{ Pa}$; the middle curve, obtained with $a = 1.8 \text{ cm}^2$, has $\overline{\Delta p_{\text{jp}}} = 630 \text{ Pa}$; the highest curve, obtained with $a = 0.8 \text{ cm}^2$, has $\overline{\Delta p_{\text{jp}}} = 1160 \text{ Pa}$. The middle setting suppresses $\dot{M}_{2,0}$, as shown in **a**.

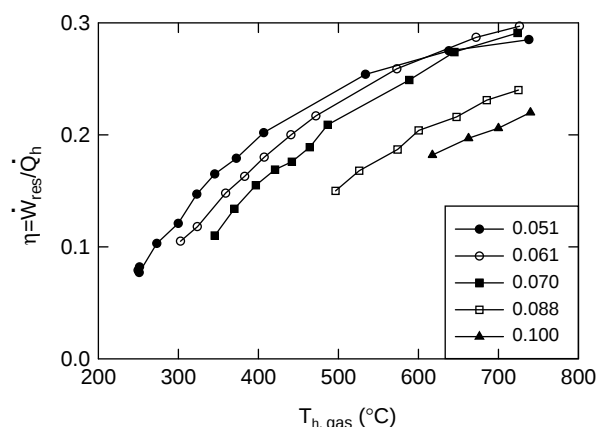


Figure 3 Thermal efficiency of the engine as a function of $T_{\text{h,gas}}$. To make a fair comparison with other thermoacoustic engines⁹, the thermal efficiency $\eta = \dot{W}_{\text{res}}/\dot{Q}_h$ is reported in terms of the power entering the resonator, $\dot{W}_{\text{res}}$, instead of the directly measured $\dot{W}_{2\text{mic}}$. A detailed computer model (ref. 14; model available at <http://rott.esa.lanl.gov/>) duplicating $\dot{W}_{2\text{mic}}$ and the magnitude of the measured pressures is used to extrapolate back to the resonator junction to determine $\dot{W}_{\text{res}}$. At the maximum $T_{\text{h,gas}}$, the difference between $\dot{W}_{2\text{mic}}$ and $\dot{W}_{\text{res}}$ is typically 4% and never more than 14%. During these measurements, the cooling water in the main cold heat exchanger is typically 15°C . The maximum efficiency obtained corresponds to 42% of theoretical maximum Carnot efficiency².

With $\bar{M}_2$ suppressed, the performance of the engine is measured as a function of p_{1c} and the temperature of the helium immediately below the hot heat exchanger, $T_{h, \text{gas}}$. Figure 3 shows the thermal efficiency², $\eta = \dot{W}_{\text{res}}/\dot{Q}_h$, where $\dot{W}_{\text{res}}$ is the acoustic power delivered to the resonator. The maximum thermal efficiency of $\eta = 0.30$ occurs near $|p_1/p_m| = 0.06$ at $T_{h, \text{gas}} = 725^\circ\text{C}$ with the engine delivering 710 W to the resonator. This corresponds to 42% of the theoretical maximum Carnot efficiency². At the most powerful operating point, $|p_1/p_m| = 0.10$, 890 W is delivered to the resonator with $\eta = 0.22$.

Although this engine already shows a large increase in efficiency over previous heat engines without moving parts, we anticipate further improvements through research on flow separation losses^{17,18} and regenerator inefficiency. We have also built one refrigerator¹⁹ according to the same principles, and we expect that it will give performance comparable to that of commercially successful refrigeration equipment. □

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Weak surface anchoring of liquid crystals

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Current nematic liquid-crystal (LC) displays rely on voltage-induced reorientation of the director (the average molecular direction) within the bulk of the LC layer. In these devices, the surface region of the LC is strongly anchored to the cell walls and does not undergo reorientation at normal operating voltages. This situation is not optimal and indeed modelling has shown that weak anchoring of the LC can in principle lead to lower operating voltages and improved steepness in the electro-optic response¹. Achieving weak anchoring in practice has proved difficult. Soft rubbing of a polymer² or photoinduced ordering of a polymer³

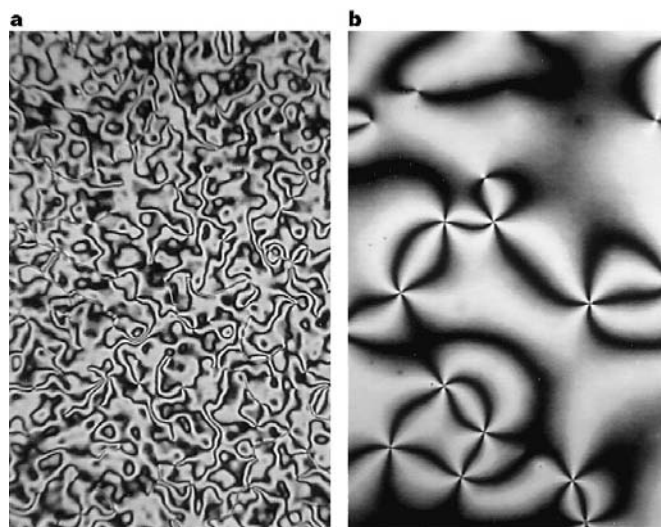


Figure 1 Photographs of two cells (between crossed polarizers). **a**, Pure nematic; **b**, nematic and oligomer (4% MXM035). The width of photographed region is 1.25 mm in both cases. Both cells were filled at 65 °C (in the isotropic phase of E7) to avoid flow alignment effects.

coating the cell walls can lead to weak azimuthal (in-plane) anchoring, but a memory effect is still present which prevents high-speed surface reorientation. Some surface treatments, such as obliquely evaporated silicon oxide, can also induce weak anchoring, but only for a restricted range of temperatures^{4,5}. Here we report a different approach to weak anchoring, which relies on the addition of small percentages of oligomeric molecules to the LC. This approach results in very small zenithal (out of substrate plane) and azimuthal (in plane) anchoring energies. When applied to nematic displays, such treatments lead to a halving of the operating voltage.

Oligomer chains were formed in a LC using the following method. A two-part photopolymer (MXM035, Merck) was added to the nematic LC E7 (Merck) at a concentration of 4%. Curing of MXM035 is known to lead to the formation of oligomers with a wide range of molecular masses, as it is a thiolene system which undergoes step growth and chain growth polymerization. In this case, curing was induced by illumination from a mercury lamp; the resulting exposure energy (all lines) at the sample was 1.0 J cm⁻². Such mixtures were then used to fill 5-μm-thick LC cells which contained amorphous polymer surfaces made from polyimide (Probimide 32, Ciba Geigy). In practice, the large-molecular-mass units formed by chain-growth polymerization phase separate from the LC during exposure, and do not fill the cell.

Figure 1 shows photographs of the cells positioned between crossed polarizers. The first cell (Fig. 1a) was filled with pure E7 and shows a random alignment texture; this is expected, as the polymer has no preferred direction. The director was parallel to the surface at all positions. The second cell (Fig. 1b) was filled with E7 + 4% MXM035 (cured before cell filling). The domain size in this second cell was clearly much larger than that seen in Fig. 1a (350–1,000 μm compared to 50–200 μm). Furthermore, the addition of the oligomer allowed very high mobility of the domains in response to LC flow, while the domains shown in Fig. 1a were fixed. In the case of the pure LC, cooling into the nematic phase after filling leads to a layer of strongly bound molecules on the surface. This is the surface memory effect⁶. The addition of the oligomer has appeared to screen out this memory effect, and has led to a 'slippery surface' condition. Domain mobility can be observed for pure nematics, but only at temperatures very close to the nematic–

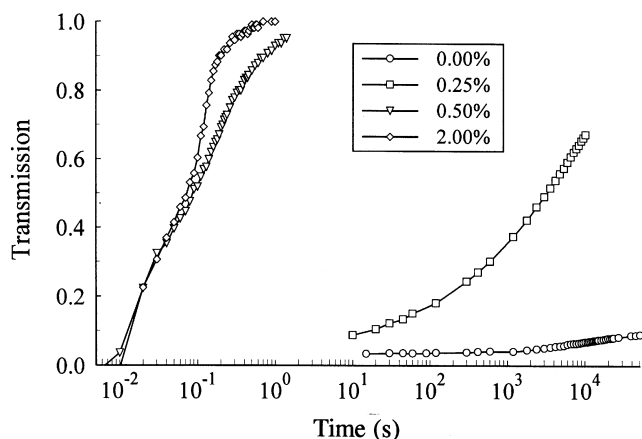


Figure 2 Transmission between crossed polarizers after removal of a 500 V r.m.s. in-plane field for various percentages of FC430 in E7. A transmission of 1.0 corresponds to a structure with the natural twist (90°). Cells were prepared with a $4.2\text{-}\mu\text{m}$ cell gap and a $500\text{-}\mu\text{m}$ electrode spacing. In each case, the cell was heated to 75°C for 5 min, then an in-plane field of 500 V r.m.s. was applied. Next, the cell was cooled slowly through the isotropic-to-nematic transition (at 58°C) while maintaining the field.

isotropic phase transition when the nematic order parameter is low. This suggests that the oligomer additives could be influencing the surface order parameter of the LC.

The next step was to perform accurate measurements of the effect of the oligomers on the surface-anchoring energy of the LC. Anchoring can be defined by two parameters⁷. The energy per unit area required to rotate the near-surface director by 90° out of the plane of the substrate is the zenithal anchoring energy (W_θ), while the energy per unit area for a 90° rotation in the substrate plane is the azimuthal anchoring energy (W_ϕ). On a flat amorphous surface, W_ϕ is zero. However, free azimuthal rotation of the director is hindered by the surface memory effect. The strength of this memory effect can be measured by cooling a chiral LC from its isotropic to its nematic phase while maintaining a large in-plane field⁶. Removal of the field then leads to relaxation from a uniform to a twisted texture at a rate which is dependent on the surface memory. The substrate surfaces were again flat polymer layers of polyimide. These were filled with E7 doped with 0.15% R1011 (Merck) to induce chirality. In this experiment, a different oligomer was used (Fluorad FC430 from 3M; a coating additive based on fluoroaliphatic polymeric esters). This material was designed to have a larger surface affinity than MXM035, and so was expected to be effective in lower concentrations. Figure 2 shows the transmission after the removal of the in-plane field (at 30°C). In the case of the LC with no oligomer, a very slow rise in transmission (and hence twist) was observed which is consistent with a strong surface memory. The addition of only 0.25% FC430 led to a larger and faster rise, while the addition of 0.5% or 2.0% of this compound led to even faster rises in transmission with rise times well under one second. These results show that the azimuthal gliding is over 5 orders of magnitude faster when small percentages of oligomer are added to the LC. This corresponds to an enormous reduction in the surface memory.

The effect of FC430 on zenithal anchoring was assessed by measurement of the voltage at which the LC is fully realigned by an out-of-plane field. This saturation voltage (V_s) is linked (at large V_s) to W_ϕ by¹:

$$W_\phi = \frac{V_s \sqrt{\epsilon_0 \Delta \epsilon K}}{d} \quad (1)$$

where d is the cell gap, $\Delta \epsilon$ is the dielectric anisotropy of the nematic

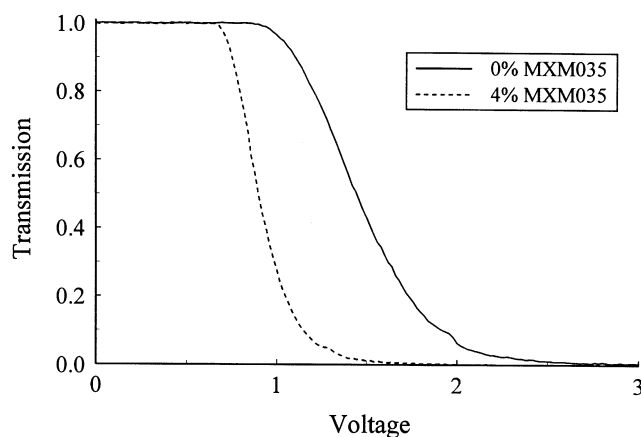


Figure 3 Transmission between crossed polarizers as a function of voltage (r.m.s. 1 kHz) for two grating-aligned twisted nematic cells. The cells were filled with pure MLC 11100-000 (solid line) or MLC 11100-000 + 4% MXM035 (dashed line). Cell gaps were $4.5\text{-}\mu\text{m}$ in both cases. Grating surfaces were formed in photoresist (Shipley 1805) by contact photolithography, to give a pitch of $1.0\text{-}\mu\text{m}$ and a depth of $0.5\text{-}\mu\text{m}$. Good-quality alignment was obtained in both cells, and a LC twist of 90° was confirmed in both cases.

and K is the average of the splay and bend elastic constants. $\Delta \epsilon = 13.9$ and $K = 13.7\text{ pN}$ for E7. Measurement of V_s was performed in cells consisting of ITO (indium tin oxide) electrodes with polyimide overlayers. Table 1 lists the results for various concentrations of the oligomer, FC430. It is clear that the addition of FC430 led to a dramatic decrease in V_s . W_ϕ on the polyimide surface is about an order of magnitude lower with 2% FC430 compared to pure E7. It is also much weaker than the anchoring measured on a wide variety of surfaces⁸. The average tilt angle of the LC induced by the surfaces was also assessed by low- and high-voltage capacitance measurements (Table 1). The pure E7 cell had a tilt of 0.0° as expected, but a monotonic rise was noted for additions of FC430. If the FC430 is lowering the surface order parameter as suggested earlier, then there will exist a surface-order electric polarization which can be minimized by the creation of a tilted near-surface layer⁹. As equation (1) was derived for the case of zero pretilt, the accuracy of W_ϕ for the samples containing FC430 is probably reduced.

The above measurements have shown that the addition of oligomers dramatically reduces the surface memory effect while also decreasing the zenithal anchoring energy. We propose that the oligomers mix with the LC in the vicinity of the surface. A molecular mass gradient would be formed in accordance with polymer solution thermodynamics¹⁰ with higher-molecular-mass oligomers closer to the surface region. The significant dilution of the nematic near the cell walls could then lead to a lowering of the order parameter. Direct measurements of surface order parameter in the presence of oligomers have not yet been performed, but initial assessment shows no change in birefringence for cells as thin as $0.2\text{-}\mu\text{m}$. Also, a 2% mixture of FC430 is sufficient to screen out the

Table 1 Anchoring properties of LC/oligomer mixtures

Mixture	Cell gap (μm)	V_s (V)	W_θ (J m^{-2})	Surface tilt ($^\circ$)
E7	3.66	141	1.6×10^{-3}	0.0
0.5% FC430 in E7	3.73	41	4.6×10^{-4}	7.4
1.0% FC430 in E7	3.77	13	1.4×10^{-4}	16.0
2.0% FC430 in E7	3.64	11	1.3×10^{-4}	21.4

Results from several cells with different percentages of oligomeric additive, showing saturation voltage (V_s), zenithal anchoring energy (W_θ) and surface tilt. V_s was recorded as the r.m.s. voltage at which all in-plane optical retardation was lost.

aligning effect of a rubbed polymer surface which is known to align via an anisotropic Van der Waals interaction¹¹. Hence reduction of order parameter might be occurring in a surface layer with a thickness of 3–200 nm. (Reduction in surface order parameter is also responsible for the weak anchoring observed on SiO surfaces at high temperatures⁵.)

We now consider the application of oligomeric additives to the twisted nematic LC mode which is widely used in active matrix displays. As these additives can screen out the alignment on rubbed polymer surface, we have instead used grating surfaces to induce a preferred surface direction¹². Cells were formed from two grating surfaces constructed so that the grooves on one surface were orthogonal to those on the other in order to induce a 90° twisted structure. Figure 3 shows the electro-optic response of two cells, one of which includes a 4% addition of MXM035, cured before cell filling. In this case, the LC used was MLC11100-000 (Merck). The cell with the oligomer reaches 10% transmission at only 1.16 V, compared to 1.87 V for the cell filled with pure LC. Such voltage reductions in a display would allow operation with only 38% of the power consumption as this is roughly proportional to the square of the operating voltage. The oligomer treatments also allow a much higher optical contrast between the 'on' and 'off' states. Weak zenithal anchoring is also expected to lead to a decrease in switch-on time and an increase in switch-off time¹. For the cell with pure LC, $t_{\text{on}} = 8.3$ ms and $t_{\text{off}} = 21.2$ ms (0–3 V, 10% to 90% transmission) while for the cell with the oligomer, $t_{\text{on}} = 6.0$ ms and $t_{\text{off}} = 34.1$ ms. This behaviour is consistent with weak anchoring.

Long, flexible, substituted hydrocarbon or fluorocarbon chains with molecular masses in the range of 1,000–10,000 Da are capable of inducing a 'slippery surface' effect in a nematic LC. One synthesis method is the reaction of a dithiol-diene model system which leads to linear oligomers. The oligomeric additives studied here contain conductive impurities, which is undesirable in many LC devices. More importantly, the treatments are found to screen out the alignment on a rubbed polymer surface and so are not compatible with current LC devices. Therefore new additives are required, which have a well controlled molecular mass and optimum surface affinity in order to reduce the surface order parameter without screening out the anisotropic Van der Waals interaction which induces alignment on a rubbed polymer surface. 'Slippery surface' additives are already showing useful results when used in conjunction with emerging LC technologies such as bistable twisted nematics, bistable cholesterics, ferroelectrics, antiferroelectrics and bistable alignments. □

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Epitaxial diamond growth on sapphire in an oxidizing environment

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Thin films of diamond are of interest for technological applications such as hard coatings, heat sinks in electronic devices and miniaturized vacuum diodes^{1–4}. They are typically produced by chemical vapour deposition, and the presence of atomic hydrogen has been considered crucial for the growth of the diamond crystals^{5–9}. Some studies have claimed diamond film growth in a hydrogen-free environment^{10–13}, but questions remained about the growth conditions in those cases. Here we report the nucleation and growth of diamond by vapour deposition in a hydrogen-free, pure oxygen environment to form crystals that are hetero-epitaxially aligned on a single-crystal sapphire substrate. In other words, we are able to achieve diamond growth under conditions

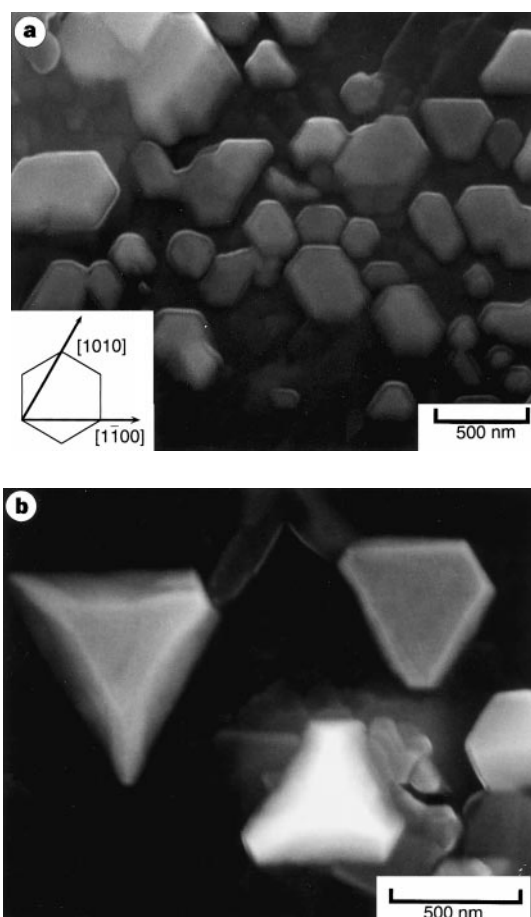


Figure 1 SEM micrographs of the films deposited at 550 °C on the ultrasmooth sapphire (0001) substrate. KrF laser ablation of graphite was employed, in 0.15 torr of oxygen. The hexagonal and well-faceted morphology of the diamond crystals are seen clearly in **b**. The inset in **a** shows the orientation of sapphire (0001) substrate, which has an hexagonal structure.

where the oxidative 'etching' of carbon must compete with its deposition. By choosing a temperature range that results in preferential oxidation of non-diamond (graphitic) carbon species to that of diamond, we are able to achieve the accumulation of diamond.

The thin-film technique used here is a pulsed laser deposition process in which a pulsed ultraviolet (UV) excimer laser is used for ablation of a solid target in order to provide highly excited vapour species that are transported onto a substrate. The pulsed laser deposition method has been intensively applied to the growth of high-quality thin films of oxide at relatively low substrate temperatures^{14,15}. The film growth rate can be controlled by changing the UV laser pulse frequency. Some attempts to grow diamond by pulsed laser deposition using graphite targets in a hydrogen atmosphere have been reported^{16,17}. Here we examined the possibility of hydrogen-free growth of diamond films on sapphire substrates by the pulsed laser deposition process using a graphite target under a partial pressure of oxygen.

The depositions were conducted in a conventional pulsed laser deposition chamber with a volume of $\sim 1,000 \text{ cm}^3$ evacuated to a base pressure of $<10^{-6}$ torr. A KrF excimer laser beam (wavelength, 248 nm; pulse duration, 20 ns; frequency, 5 Hz; Lambda Physik LPX100) was focused through a quartz window to give a photon flux of $3 \times 10^8 \text{ W cm}^{-2}$ impinging at an incident angle of 45° on a pyrolytic graphite target ($\sim 1 \text{ cm}$ diameter; 99.999% purity, Tokai Carbon G530) under an oxygen gas (99.9995% purity) pressure ranging from 10^{-6} to 1 torr. The laser-target interaction, limited by the width of the laser beam to an area of $1 \text{ mm} \times 3 \text{ mm}$ generated a greenish-white plasma plume over the graphite target.

The substrates that we used were synthetic sapphire (single-crystal $\alpha\text{-Al}_2\text{O}_3$) (0001) wafers with a crystallographic orientation flat-edge of $[1\bar{1}00]$ (Shinkosha Co.). Sapphire substrates with high melting point, high availability and high thermal conductivity are now widely used for fabrication of the blue-light-emitting devices based on GaN films. Compared to other substrates often used for diamond heteroepitaxy, such as Ni or Pt, sapphire offers the advantage of a higher melting point and a much lower tendency to incorporate gases into its crystal structure. As atomic-scale surface smoothness of the substrate is expected to enhance the epitaxial diamond film growth¹⁸, we used ultrasmooth sapphire substrates with atomically flat terraces and atomic steps of 0.2 nm height, which are formed by annealing as-supplied substrates in 1000°C in air¹⁹. We note that the substrates used here were not abraded with diamond powder before the deposition as is typical for

other processes in order to plant seed crystals onto the substrate²⁰.

The substrates were ultrasonically cleaned with acetone and ethanol, and mechanically mounted on the temperature-controlled substrate holder. The substrate was positioned parallel to the graphite target at a distance of $\sim 2 \text{ cm}$ away. The films were characterized using field-emission scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray diffraction (XRD) and micro-Raman spectroscopy.

Experimental variables including the substrate temperature and the oxygen pressure were varied to optimize diamond film growth. It is well known that carbon deposits rapidly oxidize at temperatures higher than 600°C , forcing us to keep experimental temperatures close to or below this value. With temperature ranging from 500 to 600°C and oxygen pressure either below 0.1 torr or above 0.3 torr, we observed the growth of conductive graphitic films or the absence of film growth, respectively. We found that diamond crystals could be grown at temperatures of around 600°C under oxygen pressures ranging from 0.1 to 0.2 torr. From analysis of the gas in the chamber by quadrupole mass spectrometry, the concentrations of the contaminants H_2 gas and water vapour were determined to be less than 30 p.p.b. and 0.5 p.p.m., respectively, under 0.1 torr of oxygen atmosphere, which are far too low to allow diamond growth by the conventional growth mechanism involving hydrogen⁹.

Figure 1a and b show SEM images of the surface of the film obtained by a deposition run of 4 h at 550°C and 0.15 torr O_2 gas (oxygen mass flow rate of 13 standard $\text{cm}^3 \text{ min}^{-1}$). The inset in Fig. 1a shows the orientation of the sapphire (0001) substrate with a hexagonal corundum structure; the hexagonal-shaped and well-faceted crystals are nucleated and grown with an in-plane arrangement. It was confirmed by SEM that these crystals are azimuthally aligned with the $[1\bar{1}00]$ direction of sapphire. The diamond crystal density is about 10^8 cm^{-2} , with an average diameter of approximately $0.5 \mu\text{m}$. (A few crystallites had sizes up to $5 \mu\text{m}$, while others were as small as $0.2 \mu\text{m}$.) AFM measurements indicate that the average height of the crystallites is $\sim 0.2 \mu\text{m}$. Although the growth habit of the crystals shown in Fig. 1 is similar to that observed in (111)-oriented diamond films heteroepitaxially grown on Pt(111) substrates²¹, we have not been able to use our method to grow diamond crystals on Pt(111) substrate. The (111)-orientation of the diamond crystals prepared in the present work was confirmed by XRD, as shown in Fig. 2. These results of SEM and XRD measurements indicate that (111)-oriented diamond crystals can be grown on the sapphire (0001) substrate with an in-plane epitaxial relationship of diamond $[\bar{1}10]$ //sapphire $[1\bar{1}00]$.

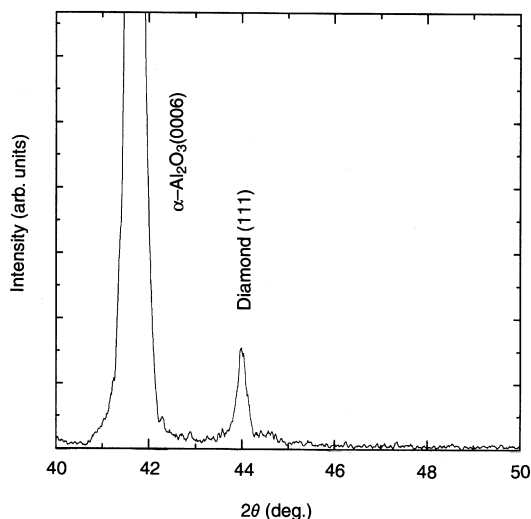


Figure 2 XRD pattern of the film shown in Fig. 1, indicating the heteroepitaxial growth of (111)-oriented diamond films.

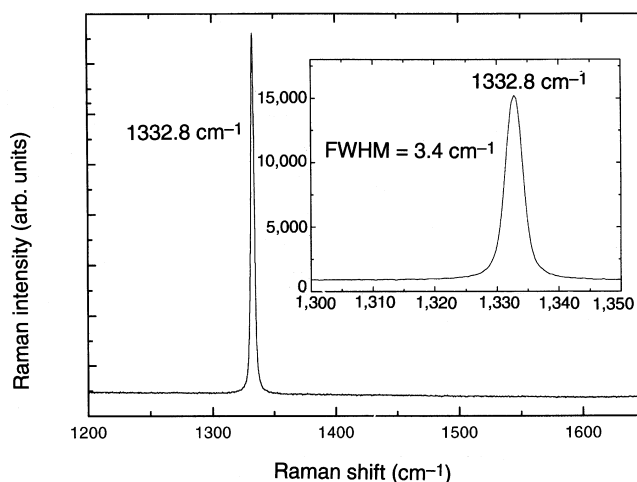


Figure 3 Micro-Raman spectrum of the crystal, indicating a peak at $1,332.8 \text{ cm}^{-1}$ that is characteristic for diamond. The inset shows the small full-width at half-maximum (FWHM; 3.4 cm^{-1}), indicative of the high-quality diamond crystal.

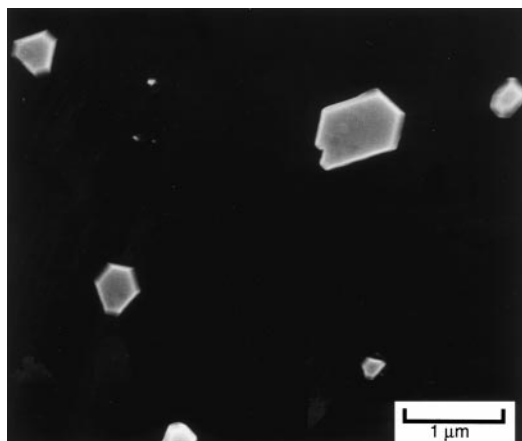


Figure 4 SEM image of the film deposited on the as-supplied sapphire (0001) substrate. Although (111)-oriented hexagonal diamond crystals have grown, the in-plane arrangement of hexagons on the surface—which was observed when ultrasmooth substrates were used (Fig. 1)—is not found.

A micro-Raman spectrometer was used for the further characterization of the deposited diamond. A laser beam of 1-μm spot size was focused onto the crystallites. Figure 3 shows the resulting Raman spectrum: it exhibits a first-order diamond peak centred at $1,332.8\text{ cm}^{-1}$. The high intensity of the diamond peak, its small full-width at half-maximum (3.4 cm^{-1}) and the small contribution of graphite phase confirm the high quality of the diamond crystal film. The Raman analysis was also carried out on deposits surrounding the diamond crystals: these spectra showed graphitic-like bands and no signal from diamond.

The diamond growth observed here, that is, in a hydrogen-free and oxidizing environment, differs from the results obtained in the earlier studies, including those using an oxygen-containing molecule in the source gas²². The present findings clearly establish that not only hydrogen, but also oxygen is able to abstract hydrogen from the growing diamond surface to form a reactive site. Our findings also establish that it is possible to grow diamond under hydrogen-free conditions, where the etching of carbon (with sp , sp^2 and sp^3 configuration) by oxygen clearly competes with vapour deposition. That is, macroscopic diamond deposition is controlled by the relative rate of these two processes, with growth occurring at temperatures where the oxidation of sp and sp^2 forms of carbon is faster than that of the sp^3 configuration present in diamond.

In order to examine the effect of substrate surface roughness on the growth of diamond, films were also deposited on an as-supplied sapphire (0001) substrate. This mirror-polished substrate had an irregular roughness of the order of 1 nm. The SEM image of a film deposited onto the as-received substrate is shown in Fig. 4. It demonstrates that reproducible growth of (111)-oriented diamond crystals with hexagonal shape can be attained on this substrate using the oxygen process but without achieving the in-plane arrangement of the hexagons on the surface obtained with the ultrasmooth substrates (compare Fig. 1).

The dependence of crystal orientation on sapphire surface roughness indicates that the in-plane arrangement of the heteroepitaxial diamond crystals is governed by steps and/or terraces present on the surface. In conventional heteroepitaxial nucleation of diamond in which hydrogen is involved, oxygen is extracted from the oxide surface resulting in degradation of crystal structure of the substrate. By contrast, the present oxygen process preserves the original crystal structure of the oxide substrate surface and we therefore expect an atomically flat heteroepitaxial interface between diamond and sapphire. On the other hand, using XRD and high-energy electron diffraction, we have found that a thin layer of (0001)-oriented

graphite is epitaxially grown on the ultrasmooth sapphire (0001) surface during the initial growth stage. The (0001) plane of sapphire has a lattice mismatch of 8.4% with the (0001) basal plane of graphite and 6.3% with the (111) diamond plane, implying that the initial formation of the strained graphite layer might be closely associated with the diamond nucleation and growth process.

To further investigate this question, it will be necessary to first study the structure of the heteroepitaxial interface to determine whether diamond nucleates directly on sapphire or on the graphite layer formed on sapphire. □

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A large and abrupt fall in atmospheric CO₂ concentration during Cretaceous times

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Marine carbonates and organic matter show a sharp increase in their ¹³C/¹²C isotope ratio at the Cenomanian/Turonian (C/T) boundary^{1,2} in the Cretaceous period. This isotopic shift resulted from an increase in the rate of sedimentary burial of ¹³C-depleted organic carbon in response to the C/T 'oceanic anoxic event'². The enhanced burial rate should have led to a significant drop

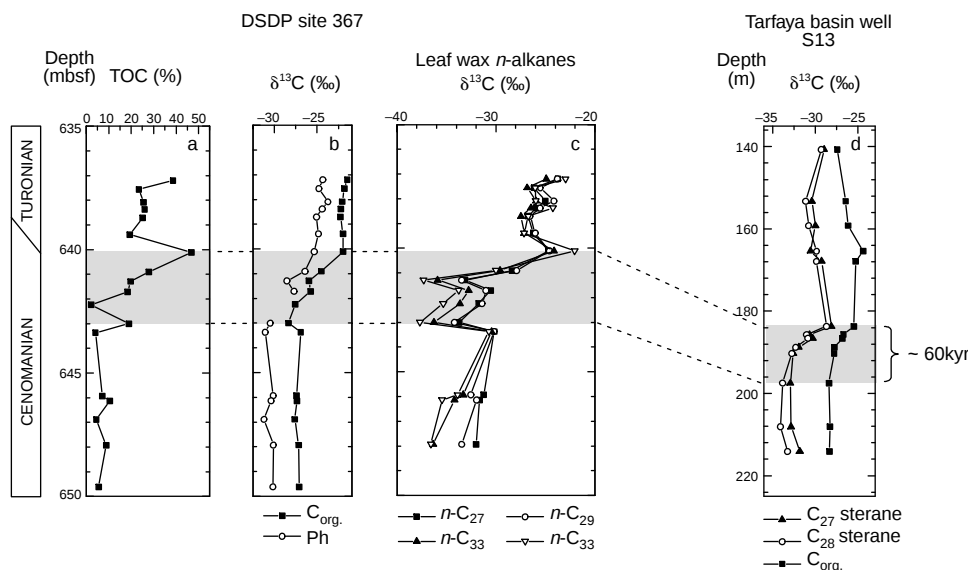


Figure 1 Bulk and biomarker data from the investigated sites; the stratigraphy is also shown. **a–c**, Data from DSDP site 367. **a**, Total organic carbon (TOC) content; **b** and **c**, carbon isotope values (in ‰ vs. PDB) of C_{org} and S-bound phytane (Ph) derived from phytoplankton chlorophyll (**b**), and leaf-wax components (**c**). **d**, Data from shelf sediments from well S13 of the Tarfaya basin; carbon isotope values of C_{org} and free steranes (C_{27} sterane (5 α -cholestane) and C_{28} sterane (5 α -24-methyl-cholestane)) derived from marine algae. The time difference (estimated

at ~60 kyr) between the onset of the $\delta^{13}C_{org}$ excursion and the first maximum carbon-isotope value for organic matter is indicated as a grey shaded area in the graphs. At site 367 carbonate carbon is absent, due to deposition below the carbonate compensation depth, but carbonate $\delta^{13}C$ values for the Tarfaya shelf sediments, although affected by diagenesis, indicate a 2.5‰ excursion (W. Kuhnt, personal communication).

in the atmospheric CO_2 concentration, which could explain the apparent climate cooling of early Turonian times^{2–4}. Here we present stable carbon isotope data for specific compounds from terrestrial leaves and marine phytoplankton, and quantify the abruptness and magnitude of the atmospheric CO_2 concentration change. Isotope shifts in leaf-wax components extracted from abyssal sediments in the northeastern tropical Atlantic Ocean—the components are wind-delivered from Africa—indicate a sudden change in plant communities of the north African continent. Specifically, the data suggest that plants using the C_3 -type photosynthetic pathway were succeeded by plants using the C_4 -type pathway. If C_4 plants can outcompete C_3 plants only at atmospheric CO_2 concentrations below 500 p.p.m.v. (ref. 5), the observed vegetation change indicates a far larger reduction in C/T CO_2 concentration—some 40–80%—than previously suggested⁶. The isotopic excursion in the marine phytoplankton compounds is consistent with this estimate. We infer that this dramatic fall in the atmospheric CO_2 concentration was abrupt, occurring in just 60,000 years.

The climate in middle–late Cretaceous times has been described as a “greenhouse climate” with minimal Equator-to-pole thermal gradients⁷. It has been suggested that this climate condition resulted from extensive periods of volcanic outgassing^{8,9} leading to increased atmospheric concentrations of the greenhouse gas carbon dioxide (estimates of the partial pressure of CO_2 (p_{CO_2}) vary between 3 and 12 times modern (pre-industrial) values¹⁰, that is, 900–3,300 p.p.m.v.). However, the widespread burial of organic matter in black shales during the C/T ‘oceanic anoxic event’ (OAE) probably led to a significant reduction in p_{CO_2} (ref. 2). Such a decrease is suggested by the drop in the isotope effect associated with carbon fixation (ϵ_p) by phytoplankton in the Cretaceous Western Interior Seaway of North America⁶. This decline is also documented indirectly by oxygen-isotope data, which indicate an 8–13 °C cooling at high latitudes during the early Turonian^{2,3}. Additional indications of global cooling are provided by micropalaeontological data, showing an incursion of boreal fauna into low-latitude seas⁴.

Here we present the results of a compound-specific carbon-isotope study of C/T sediments deposited in the southern part of

the North Atlantic Ocean off the coast of northwest Africa (Deep Sea Drilling Project (DSDP) site 367, close to the Equator). The laminated black shales consist of a mixture of terrigenous silicates and clay minerals¹¹, high amounts of organic matter (up to 50 wt%) and some biogenic carbonate. The thermally immature organic matter is almost exclusively of marine origin, as revealed by the Rock Eval hydrogen indices¹² and by the extremely low abundance of lignin pyrolysis products generated from the kerogen. The saturated hydrocarbon fractions consist mainly of short-chain n -alkanes (with no obvious odd-over-even carbon number predominance), isoprenoids of bacterial/algal origin, and long-chain n -alkanes with a strong odd-over-even carbon number predominance (Carbon Preference Index CPI = 2.7–5.1) derived from leaf waxes of higher plants¹³. An important mode of transport of leaf-wax n -alkanes is via wind, as indicated by the high relative amounts of leaf-wax components in aeolian dust collected at remote ocean sites¹⁴; consequently, they are common constituents of extractable organic matter of recent and ancient abyssal plane sediments¹⁵. Taking into account the prevailing wind direction, the most likely candidate for the origin of these wind-transported terrestrial n -alkanes is the nearby north African continent. Often, the generation of n -alkanes from other sources during diagenesis and catagenesis makes carbon-isotope analyses of the leaf-wax lipids impossible. In the C/T sediments described here, the leaf-wax components are still relatively abundant in the hydrocarbon biomarker fraction due to the fact that most marine biomarkers were transformed to sulphur-bound components during early diagenesis¹⁶.

The isotopic compositions of the terrestrial n -alkanes show a remarkable shift coincident with the positive isotope-ratio excursion observed here for organic carbon ($\delta^{13}C_{org}$; Fig. 1b) and worldwide^{1–3} for marine carbonates (~2.5‰). Most terrestrial plants do not show the pronounced dependence of ϵ_p values on p_{CO_2} that is observed for marine phytoplankton⁶; this results from the ability of plants to regulate the amount of CO_2 that enters their leaves by adjusting the stomatal density of the leaves^{17,18}. ϵ_p values of terrestrial higher plants can vary with humidity^{17,18} but, with the exception of extreme environments, such changes are expected to cause only minimal isotopic variability. Therefore, leaf-wax n -

alkanes are expected to increase by only $\sim 2.5\%$ in response to the changes in $\delta^{13}\text{C}$ values of atmospheric/oceanic CO_2 during the C/T OAE^{1–3}. However, for the leaf-wax *n*-alkanes a far larger shift (of 10–16‰) is observed (Fig. 1c). The magnitude of the isotopic shift of the sedimentary *n*-alkanes is consistent with a change in the source of these leaf-wax lipids from C_3 - to C_4 -type plants. The carbon isotope effect differs between the two photosynthetic pathways, such that $\delta^{13}\text{C}$ values for bulk plant material range from -22% to -30% for C_3 and from -10% to -14% for C_4 (ref. 19). The *n*-alkanes are typically depleted in ^{13}C relative to the bulk material by 6‰ and 10‰, respectively²⁰.

In the lower part of the studied section (until 641.29 m) *n*-alkane isotopic values (-38% to -30%) are typical of a predominantly C_3 -type origin and become depleted in ^{13}C with increasing carbon number (Fig. 2). This latter trend has been previously observed for C_3 plants²⁰ and for sediment samples dominated by detritus derived from such plants²¹. In the upper part of the section, *n*-alkanes exhibit $\delta^{13}\text{C}$ values (-22% to -27%) that are indicative of mainly C_4 -type input or a mixture of C_3 -type and C_4 -type leaf-wax lipids. In marked contrast to the C_3 -dominated *n*-alkane samples, $\delta^{13}\text{C}$ values of the individual leaf-wax components in these samples are nearly constant with increasing chain length (Fig. 2), which is consistent with what has been observed for C_4 plants²⁰. The different trends of $\delta^{13}\text{C}$ values versus carbon number for samples dominated by C_3 -type and C_4 -type plants results in a larger carbon-isotope shift for longer *n*-alkanes (Fig. 1c).

Direct evidence (that is, plant remains) for the presence of C_4 -type photosynthesis in the fossil record is scarce, due to the absence of readily fossilized woody parts in most plants using the C_4 system. Instead, the $^{13}\text{C}/^{12}\text{C}$ ratios of tooth enamel of herbivorous animals, of palaeosol carbonate, and of terrestrial-derived organic matter have been used as tracers for ecosystems dominated by C_3 -type and C_4 -type plants, and suggest that the C_4 -type system did not represent an important part of the terrestrial ecosystem until the late Miocene⁵. However, on the basis of palaeo p_{CO_2} reconstructions, it was proposed that a C_4 -type pathway could have evolved independently during the Carboniferous–Permian glaciation and subsequently disappeared in the Mesozoic⁵, while other authors^{22,23} suggest that C_4 -type photosynthesis could have evolved earlier than the late Miocene. Carbon-isotope measurements provide additional evidence for the presence of C_4 -type plant remains in pre-Miocene sediments. Such examples include Carboniferous calcified root mats²⁴, a late Cretaceous lignite sample²³, collagen from dinosaur bones²³, and *n*-alkanes in extracts from the Eocene Green River Formation²⁰. But until now, evidence for a CO_2 -driven shift from a C_3 -type to a C_4 -type dominated ecosystem has not been observed for sediments older than late Tertiary.

The dominant photosynthetic pathway for a given ecosystem (C_3 or C_4) depends on both p_{CO_2} and temperature (Fig. 3, refs 25, 26).

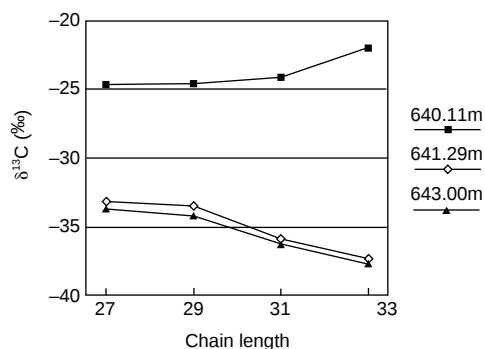


Figure 2 $\delta^{13}\text{C}$ values of leaf lipids versus chain length. The $\delta^{13}\text{C}$ values of the individual leaf-wax components for a sample before (643.0 m), during (641.29 m) and at the first maximum of (640.11 m) the $\delta^{13}\text{C}_{\text{org}}$ excursion indicate the rapid shift in the source of these higher plant lipids from C_3 - to C_4 -type plants.

Plants using the C_4 photosynthetic pathway use an active CO_2 -concentrating mechanism that provides them with an advantage over C_3 plants at low CO_2 levels and high temperatures. But at higher CO_2 levels the C_4 pathway becomes less advantageous, due to the higher energy demand for the concentrating mechanism. Consistent with this, a 10‰ shift in $\delta^{13}\text{C}$ values was reported for *n*-alkanes in Pleistocene high-altitude lake sediments and attributed to a change from C_3 to C_4 plants resulting from lower p_{CO_2} levels during glacial times¹⁹. Assuming that equatorial temperatures were comparable to present-day temperatures, as is suggested by oxygen-isotope data for the Cenomanian²⁷, and a similar response to CO_2 partial pressure for Cretaceous C_3 -type/ C_4 -type plants, p_{CO_2} during the C/T OAE would have been no more than twice that of the modern (pre-industrial) atmosphere. In fact, Cerling *et al.*⁵ suggested that the C_4 photosynthetic pathway would not be present at atmospheric CO_2 levels of more than 500 p.p.m.v. Assuming that CO_2 levels for the middle Cretaceous ranged from 3 to 12 times modern values¹⁰, this suggests that p_{CO_2} decreased by 40–80%.

In addition to low p_{CO_2} , aridity can also lead to a competitive advantage for C_4 plants²⁸, as low humidity will lead to stomatal closure and a decreased intercellular CO_2 level²⁶. But at the high p_{CO_2} levels suggested for the Cretaceous, C_3 -type plants will predominate independent of the level of humidity^{5,25}. Increased aridity of the north African continent could have facilitated an increase in C_4 -type vegetation during the C/T OAE when p_{CO_2} was significantly lower (that is, below 500 p.p.m.v.). The presence of both marine and terrestrial biomarkers in sediments from this section provides an opportunity to compare the timing and nature of the carbon-isotope excursion on land and in the marine environment. ϵ_{p} values for marine phytoplankton show a pronounced dependence on CO_2 concentration: therefore a drop in CO_2 levels should be reflected in their $\delta^{13}\text{C}$ values. For the Western Interior Seaway of North America, a geoporphylin fraction was used to calculate a 1.5‰ decrease in ϵ_{p} values during the C/T OAE²⁹, and this was subsequently interpreted as a $\sim 20\%$ reduction in p_{CO_2} levels⁶. In sharp contrast, at site 367 the 6‰ increase in the $\delta^{13}\text{C}$ values for sulphur-bound phytane derived from algal chlorophyll (Fig. 1b) records a 3.5‰ decrease in ϵ_{p} values, assuming a 2.5‰ excursion for

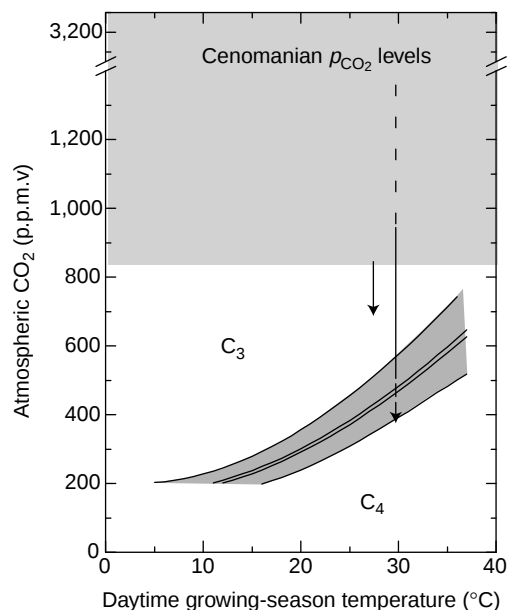


Figure 3 The C_3/C_4 crossover function. This function shows the p_{CO_2} dependence of the C_3/C_4 crossover temperature (after ref. 25). The estimated range of Cenomanian p_{CO_2} levels is indicated as a grey shaded area. The drop in p_{CO_2} levels that is observed here (large broken arrow) and the previously⁶ reported drop (small arrow) for the C/T OAE are shown. It is assumed that equatorial temperatures were comparable to present-day values²⁷.

inorganic carbon¹⁻³. This larger decrease in ϵ_p values is also suggested by the 5–6‰ increase in $\delta^{13}\text{C}$ values observed for a multitude of phytoplankton biomarkers from two other North Atlantic C/T sites (that is, the Tarfaya Atlantic coastal basin (Morocco; Fig. 1d) and DSDP site 144 off the coast of northern South America (M.M.M.K. *et al.*, unpublished results)). It is difficult to determine which marine site best records the global p_{CO_2} change during the C/T OAE, as physiological and environmental variables other than p_{CO_2} can affect ϵ_p values for marine phototrophs. However, the larger decrease in ϵ_p values observed for these three North Atlantic sites is consistent with the 40–80% decrease in p_{CO_2} required for a transition from C₃-type to C₄-type plants.

There is a difference in timing of the isotope excursion between the marine-derived organic matter and phytane, and the terrestrial *n*-alkanes (Fig. 1). The magnitude of this time lag can be determined if the time difference is known between the onset of the $\delta^{13}\text{C}_{\text{org}}$ excursion and the first maximum carbon-isotope value for organic matter. As the $\delta^{13}\text{C}$ excursion is synchronous for all sites³⁰, data from the expanded C/T section of the Tarfaya basin^{31,32} can be used for this calculation. An accumulation rate of $\sim 120 \text{ cm kyr}^{-1}$ (ref. 31) has been derived for these sediments, leading to a calculated duration for this particular excursion interval of $\sim 60 \text{ kyr}$ (Fig. 1). Assuming a constant accumulation rate, the difference in timing between the onset of the carbon-isotope excursion of marine markers and the $\delta^{13}\text{C}$ shift for terrestrial markers is $\sim 30 \text{ kyr}$. This difference could be explained by the time needed to reach the low p_{CO_2} values that are favourable for a C₄-type pathway through the burial of organic matter.

The enhanced burial of organic matter during the C/T OAE led to a profound decrease in p_{CO_2} (ref. 2), allowing the appearance of plants using a CO₂-concentrating mechanism (C₄-type) in the Cretaceous north African plant community. This shows that in addition to anthropogenic emissions, perturbations of the biogeochemical cycles may have an abrupt and dramatic effect on atmospheric CO₂ concentrations. □

Methods

Bulk analyses. Total organic carbon (TOC) contents were determined by a LECO C/S analyser. The $\delta^{13}\text{C}$ values ($\pm 0.1\text{‰}$) ($\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}} / ({}^{13}\text{C}/{}^{12}\text{C})_{\text{standard}}] - 1$; the Pee Dee belemnite standard (PDB) has been used) were measured on bulk sediments using automated on-line combustion after removal of the inorganic carbonates with diluted HCl.

Compound-specific analyses. Powdered samples (15–30 g) were Soxhlet extracted for $\sim 24 \text{ h}$ to obtain the total lipid fraction. An aliquot ($\sim 250 \text{ mg}$) of the total extract was separated into an apolar and a polar fraction using column chromatography. AgNO₃ column chromatography and urea adduction were used to obtain *n*-alkane fractions. The hydrocarbons that were released from the polar fraction by Raney nickel desulphurisation and subsequent hydrogenation were isolated using column chromatography. Samples were analysed by gas chromatography-mass spectrometry (GC-MS) for identification. Compound-specific $\delta^{13}\text{C}$ analyses were performed using GC-isotope-ratio mass spectrometry (GC-IRMS). The $\delta^{13}\text{C}$ values for individual compounds are the means of duplicate runs ($\sigma = \pm 0.3$ to 0.6) expressed versus PDB.

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Large-scale chemical and thermal division of the Pacific mantle

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Isotope analyses of mid-ocean-ridge basalts have led to the identification of large-scale geochemical provinces, with a clear distinction between the Pacific and the Atlantic or Indian Ocean basins^{1,2}. It is widely believed that Pacific ridges are formed from a single, fairly well mixed mantle reservoir³, extending from the Australian–Antarctic discordance to the Juan de Fuca ridge and representing one of the largest chemically coherent mantle domains on the Earth^{4,5}. However, the evidence for this conception is mostly based on samples from the northern Pacific ridges. Here we report Sr, Nd and Pb isotope data from the Pacific

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Antarctic ridge that reveal different isotopic signatures north and south of the Easter microplate (25° S). The evidence for two large-scale geochemical domains is further strengthened by the observation of different average depths of the ridge axes north and south of the 25° S boundary. This boundary is located at the southeastern end of the Darwin rise/Pacific Superswell area⁶, which is interpreted as a zone of upwelling⁷ from the lower mantle that has persisted since Cretaceous times. We propose that this upwelling has led to the separation into two mantle domains with their own convective histories, producing slight differences in their average isotopic signatures and thermal regimes.

Two cruises sampled the Pacific Antarctic ridge (PAR) between 53° S and 66° S (RV *Melville* in 1994 and RV *L'Atalante* in 1996) providing constraints on the structure of the mantle along 4,000 km of southern Pacific sea floor. Isotope data from 53–57° S have been recently reported by Castillo *et al.*⁸. We report here data from 56–66° S (Fig. 1 and Table 1). Following conventional isotopic representations, the data have been plotted in $^{143}\text{Nd}/^{144}\text{Nd}$ versus $^{87}\text{Sr}/^{86}\text{Sr}$, and $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$, diagrams (Fig. 2). For comparison, we have compiled an updated database of the Pacific spreading system (see Supplementary Information for references, and Fig. 2 legend for the adopted standard values).

Compared to most East Pacific Rise (EPR) samples north of the Easter microplate, the PAR 53–66° S samples generally plot on low Sr and high Pb arrays in Nd–Sr and Sr–Pb isotopic plots, respectively. This trend is also observed for EPR basalts between 30° and 35° S (refs 9, 10), from the north Chile ridge¹¹, and from the southeast Indian ridge (SEIR) east of the Australian–Antarctic discordance (AAD)⁵. In order to map this peculiar isotope signature along the Pacific spreading system, we draw reference lines in Nd–Sr and Sr–Pb plots, which correspond to the mean Pacific spreading

system arrays (including all samples) as defined by our new compilation (the equations of these lines are given in Fig. 2 legend). Then we calculate the vertical deviation of data points from the reference lines (denoted $\delta(\text{Nd–Sr})$ and $\delta(\text{Sr–Pb})$ for Nd–Sr and Sr–Pb isotope plots, respectively). Points falling above the reference lines have positive deviation (green squares in Fig. 3), those falling below have negative deviation (red squares in Fig. 3).

Using this approach, three observations can be made. The first is that along the Pacific spreading system, with the exception of Gorda and Juan de Fuca ridges, there is quite a good agreement between positive and negative $\delta(\text{Nd–Sr})$ and $\delta(\text{Sr–Pb})$ distributions. Positive and negative values are generally well distributed on either side of a boundary located at 25° S (Easter microplate), delineating two large-scale provinces (Fig. 3). The southern province extends from the AAD up to 25° S including the north Chile ridge, whereas the northern province encompasses the EPR north of 25° S and the Galapagos spreading centre. The southern domain is defined assuming that the intervening unsampled portions of the EPR, PAR and SEIR exhibit coherent isotope signature (this is supported by unpublished data for the PAR at 38° S which plot on low Sr and high Pb arrays: C. Hémond, personal communication). In the southern province, the main exceptions to the systematics occur principally along the PAR near 54° S, where the Hollister ridge is known to interact with the PAR^{8,12}, and along the south Chile ridge near 46° S, where the source of basalts is suspected to be contaminated by the nearby subduction zone¹³. Second-order exceptions also occur near 65° S, as the PAR reaches its southernmost extremity. In the northern province, exceptions are found near 21° N, off the Gulf of California.

To be sure that the two isotopic signatures seen north and south of 25° S cannot be attributed to the prevailing influence of enriched

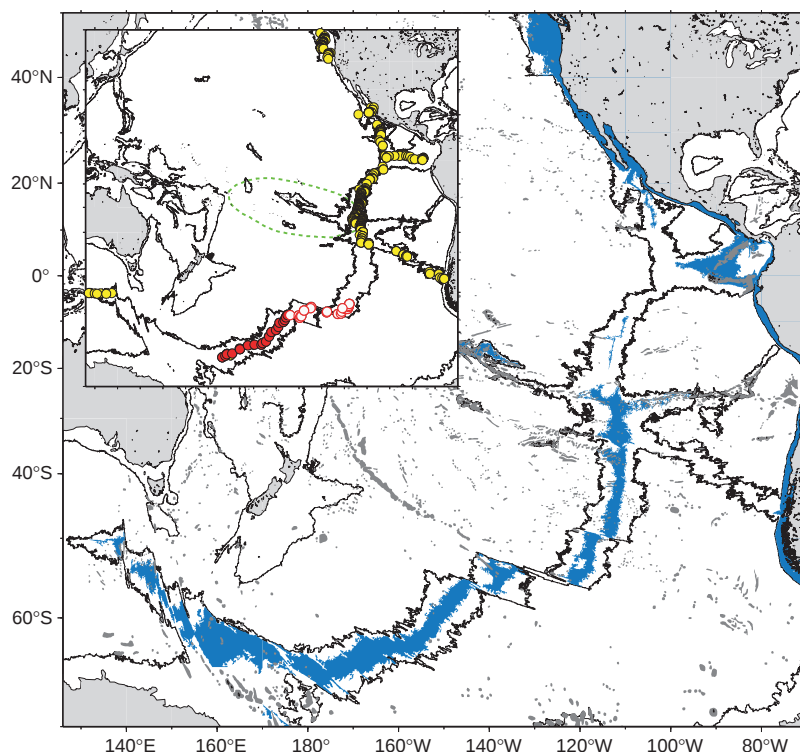


Figure 1 Bathymetric map of the Pacific spreading system. The bathymetry (sampled after the grid used in ref. 28) is derived from satellite altimetry and ship depth soundings, including the most recent multibeam data acquired along the PAR and southern EPR (refs 30, 31). The areas shown in blue are where the sea is less than 3,000 m deep; they indicate two bathymetric provinces: the deep EPR province from 20° N to 25° S, and the shallow province from the Easter microplate to the AAD. Inset, the Pacific spreading system sampling. Sampling of the PAR

from 56° to 66° S during the Pacantarctic cruise (RV *L'Atalante*, 1996) is shown in filled red circles. Samples recently reported⁸ (PAR, 57–53° S) are shown in open red circles. Other samples are shown as filled yellow circles (see Supplementary Information for references). The green dashed line indicates the South Pacific isotopic and thermal anomaly (SOPITA)¹⁴, location of the Pacific Superplume²⁰ (thought to have created the present-day Pacific Superswell^{15,16} and the Cretaceous Darwin rise^{6,17}).

mantle in one of the two domains, we have restricted the comparison to normal mid-ocean-ridge basalts only (N-MORB: defined here as all Pacific MORB except those coming from segments associated with known hotspots, namely the Galapagos spreading centre between 89° and 96° E, the east rift of Easter microplate and

the segment near the Hollister ridge–Louisville hotspot system). The northern N-MORB have on average higher Sr, higher Nd and lower Pb isotopic ratios (0.70257 ± 0.00026 (2σ), $n = 300$, 0.51314 ± 0.00009 (2σ), $n = 195$, 18.39 ± 0.37 (2σ), $n = 268$) than the southern ones (0.70248 ± 0.00022 (2σ), $n = 92$,

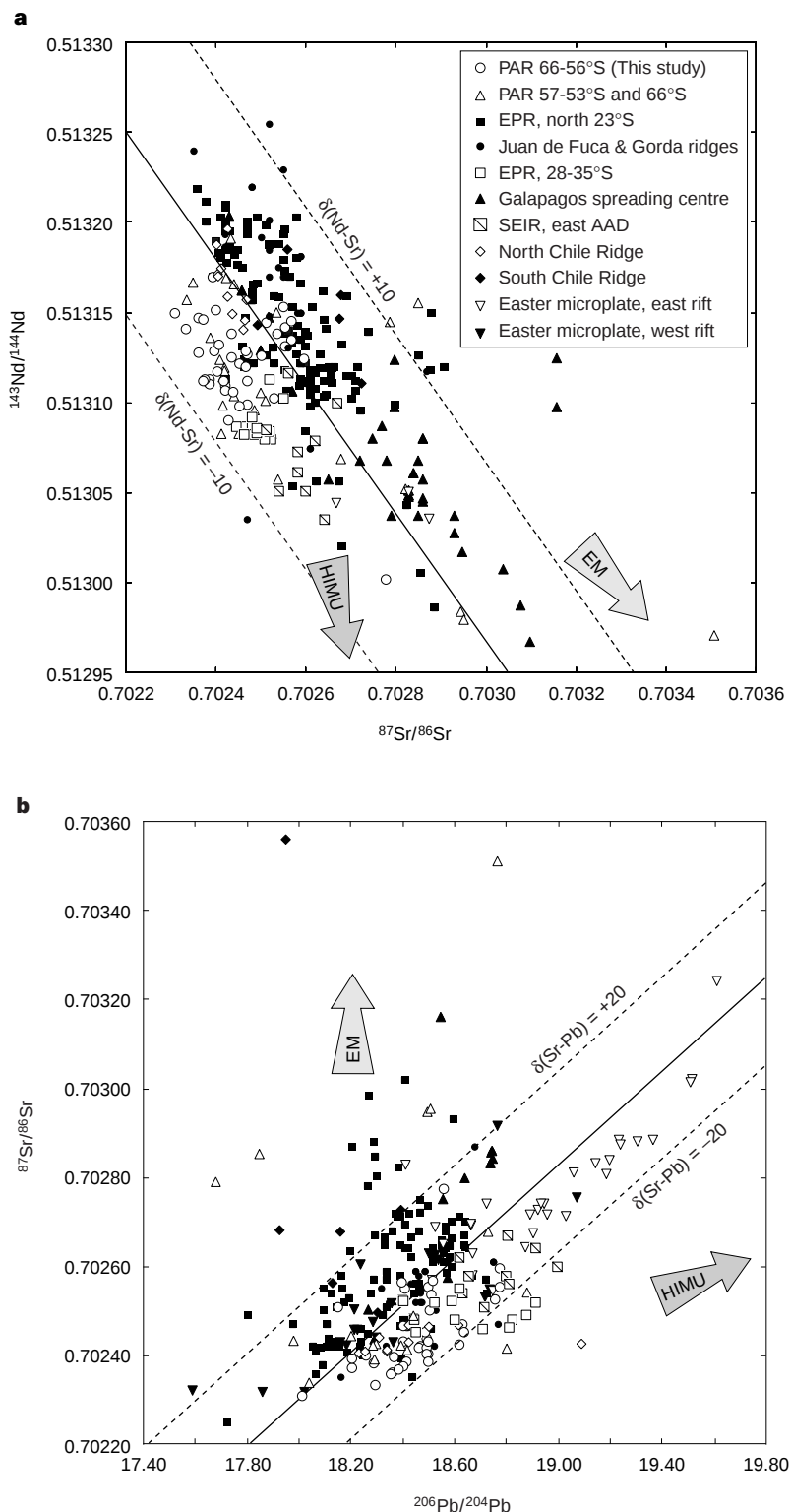


Figure 2 Sr–Nd (**a**) and Pb–Sr (**b**) isotopic plots. All data have been renormalized to equivalent standard values. Sr isotopic compositions are relative to NBS987 = 0.71025 or E&A = 0.70803. Nd isotopic compositions are relative to LaJolla Nd = 0.511852 or BCR1 = 0.512627. Pb isotopic compositions relative to NBS981; $^{206}\text{Pb}/^{204}\text{Pb} = 16.9373$, $^{207}\text{Pb}/^{204}\text{Pb} = 15.4925$, $^{208}\text{Pb}/^{204}\text{Pb} = 36.7054$. The

equations of the reference lines are $(^{143}\text{Nd}/^{144}\text{Nd}) = -0.35292 (^{87}\text{Sr}/^{86}\text{Sr}) + 0.76107$ for Nd–Sr plot and $(^{87}\text{Sr}/^{86}\text{Sr}) = 5.25 \times 10^{-4} (^{206}\text{Pb}/^{204}\text{Pb}) + 0.69286$ for Sr–Pb plot. (See Supplementary Information for references used in compiling this figure).

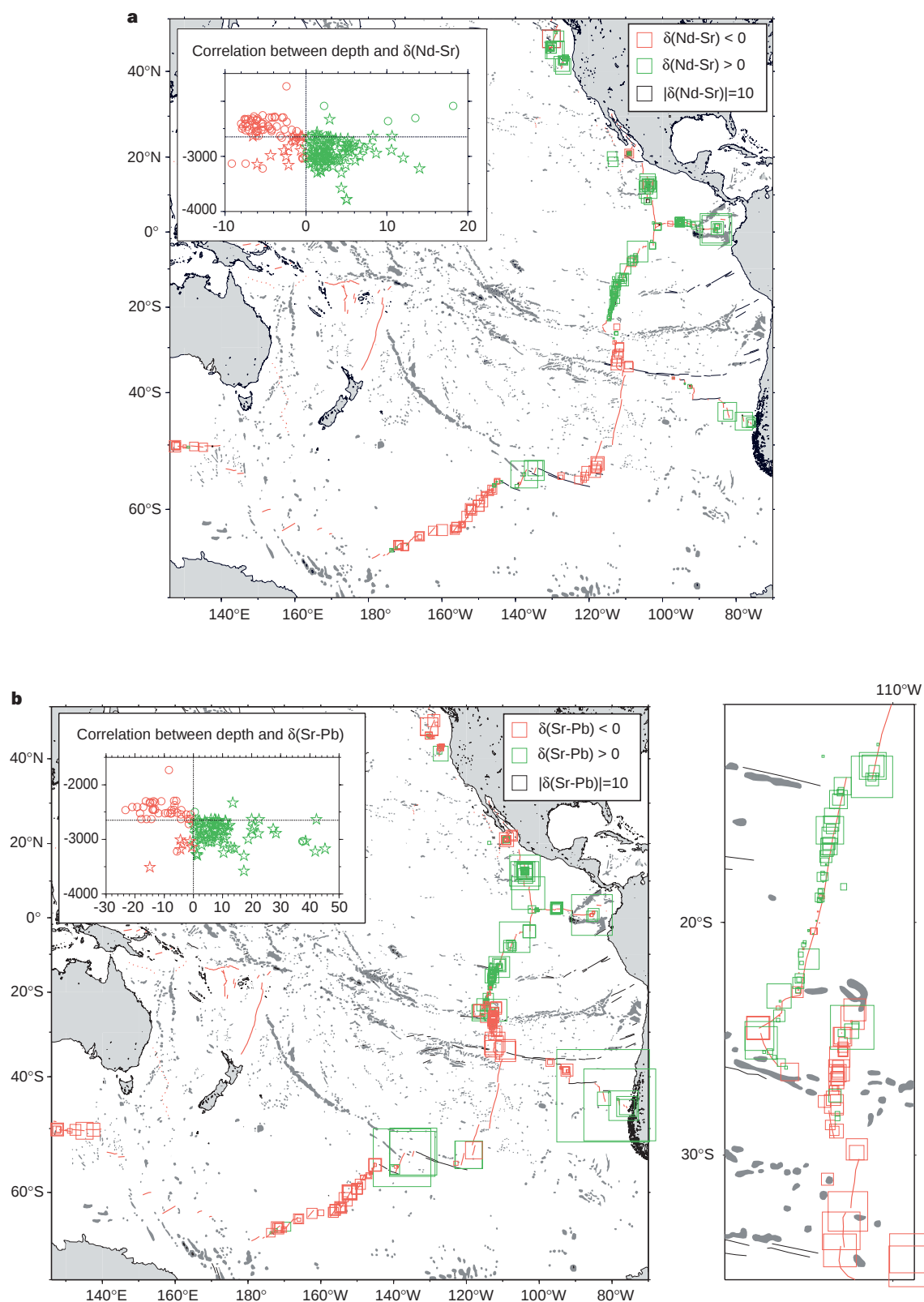


Figure 3 $\delta(\text{Nd-Sr})$ (**a**) and $\delta(\text{Sr-Pb})$ (**b**) along the Pacific spreading system. For any Pacific spreading system sample (S), the $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ deviations from the reference line (RL) are expressed as: $\delta(\text{Nd-Sr}) = [(^{143}\text{Nd}/^{144}\text{Nd})_S - (^{143}\text{Nd}/^{144}\text{Nd})_{\text{RL}}]10^5$ (Nd-Sr plot) and $\delta(\text{Sr-Pb}) = [(^{87}\text{Sr}/^{86}\text{Sr})_S - (^{87}\text{Sr}/^{86}\text{Sr})_{\text{RL}}]10^5$ (Sr-Pb plot). References for the data used in this figure are the same as used to compile Fig. 2. The colour of the square is related to the sign of the deviation: green indicates positive and red shows negative. The square size is proportional to the absolute deviation value. Spreading axis (in red) and intraplate

volcanism (in black) are shown (digitized on the map in ref. 28). An enlargement of the Easter microplate area is shown in the right panel of **b**. The insets at top left show $\delta(\text{Nd-Sr})$ and $\delta(\text{Sr-Pb})$ versus ridge depth (m) for two selected areas: the EPR 18°N–23°S (stars) and the PAR 53–66°S (circles). Depths of samples are deduced from ref. 28. Red symbols show negative $\delta(\text{Nd-Sr})$ and $\delta(\text{Sr-Pb})$ values, green symbols show positive $\delta(\text{Nd-Sr})$ and $\delta(\text{Sr-Pb})$ values. The vertical line separates negative from positive values; the horizontal line indicates the threshold depth (2,650 m) at which $\delta(\text{Nd-Sr})$ and $\delta(\text{Sr-Pb})$ change sign.

0.51311 ± 0.00008 (2σ), $n = 92$, 18.51 ± 0.48 (2σ), $n = 89$). This comparison points out that the large-scale division of the Pacific mantle is not only due to a Sr anomaly. It is most clearly seen in Sr–Nd and Sr–Pb plots (but not readily seen in Pb–Pb or Nd–Pb plots).

Pacific N-MORB have generally lower $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios, and higher $^{206}\text{Pb}/^{204}\text{Pb}$ ratios, than mid-Atlantic N-MORB^{2,4}. A similar relationship exists between the two Pacific provinces. Therefore, given the existing sampling of the global spreading system, the southern Pacific N-MORB display an end-member isotope signature (low $^{87}\text{Sr}/^{86}\text{Sr}$, $^{143}\text{Nd}/^{144}\text{Nd}$ and high $^{206}\text{Pb}/^{204}\text{Pb}$).

The second observation is that the two geochemical domains defined above correspond to two large-scale bathymetric provinces of the Pacific spreading system. 25°S is a well marked bathymetric boundary (Fig. 1): on the basis of sampling, the average axial depth is $\sim 2,850$ m in the northern province and $\sim 2,450$ m in the southern province (except for the Chile ridge)^{11,13}. There is no clear correlation between $\delta(\text{Nd–Sr})$ – $\delta(\text{Sr–Pb})$ and ridge depth within each

province, but $\delta(\text{Nd–Sr})$ and $\delta(\text{Sr–Pb})$ change sign at a threshold depth of $\sim 2,650$ m (Fig. 3). These systematics fail for samples close to the AAD or along the north Chile ridge which are too deep for their low $\delta(\text{Nd–Sr})$ and $\delta(\text{Sr–Pb})$ values. The difference in bathymetry between the two provinces is not related to a significant change in spreading rate at 25°S , nor can it be explained by differences in plate kinematic histories, as 25°S does not correspond to any geodynamic boundary. Instead, differences in the thermal structure of the uppermost mantle temperature on either side of the boundary could control large-scale variations in the ridge axial depth.

The third observation is that this boundary at 25°S is located at the southeastern end of the South Pacific isotopic and thermal anomaly (SOPITA)¹⁴, which is related to the 'South Pacific Superswell'^{15–17}. It has been suggested⁶ that the South Pacific Superswell, which extends between 10 – 30°S and 110 – 170°W , is the modern continuation of the Cretaceous Darwin rise. The Pacific Superswell

Table 1 Isotope analyses of 56–66° S PAR samples

Sample	Long. ($^\circ\text{W}$)	Lat. ($^\circ\text{S}$)	$^{87}\text{Sr}/^{86}\text{Sr}^*$	$^{143}\text{Nd}/^{144}\text{Nd}^\dagger$	$^{206}\text{Pb}/^{204}\text{Pb}^\ddagger$	$^{207}\text{Pb}/^{204}\text{Pb}^\ddagger$	$^{208}\text{Pb}/^{204}\text{Pb}^\ddagger$
CV01	173.75	65.10	0.702551	0.513153	18.408	15.562	38.154
CV01-g	173.75	65.10	0.702597	0.513124	18.772	15.606	38.633
CV02-g	172.43	64.83	0.702568	0.513135	18.395	15.491	37.916
CV03	171.88	64.53	0.702423	0.513109	18.470	15.497	37.968
CV03-g	171.88	64.53	0.702406	0.513117	18.497	15.498	38.093
CV04-g	169.40	64.40	0.702512	0.513144	18.152	15.453	37.596
CV06	166.06	63.45	0.702362	0.513128	18.358	15.498	37.899
CV06-g	166.06	63.45	0.702397	0.513129	18.366	15.493	37.894
CV07	165.96	63.54	0.702402	0.513151	18.261	15.505	37.889
CV07-g	165.96	63.54	0.702778	0.513002	18.558	15.578	38.376
CV08	162.44	62.77	0.702419	0.513112	18.464	15.496	37.974
CV09	159.61	62.66	0.702383	0.513111	18.287	15.613	38.174
DR02-g	156.54	62.64	0.702335	0.513141	18.295	15.488	37.807
DR02-1	156.54	62.64	0.702362	0.513147	18.354	15.481	37.838
DR03-1	156.08	62.32	0.702421	0.513107	18.493	15.500	38.013
DR03-2	156.08	62.32	0.702439	0.513206	18.493	15.495	37.993
DR05-g	154.54	62.0	0.702407	0.513132	18.491	15.507	37.998
DR05-3	154.54	62.0	0.702435	0.513138	18.495	15.501	37.983
DR05	154.54	62.0	0.702455	0.513122	18.483	15.494	37.953
DR06-g	153.21	60.94	0.702502	0.513126	18.504	15.505	37.987
DR06-2	153.21	60.94	0.702389	0.513113	18.496	15.501	37.970
DR07-1g	152.08	60.00	0.702472	0.513099	18.632	15.498	38.074
DR07-2g	152.08	60.00	0.702454	0.513098	18.637	15.527	38.154
DR07-1g	152.08	60.00	0.702428	0.513090	18.620	15.505	38.086
DR08-2	150.02	59.50	0.702388	0.513110	18.412	15.489	37.885
DR08-3	150.02	59.50	0.702376	0.513113	18.404	15.483	37.858
DR09-g	149.14	58.85	0.702467	0.513120	18.422	15.503	37.953
DR09-1	149.14	58.85	0.702372	0.513112	18.382	15.483	37.882
DR10-1g	148.50	57.89	0.702473	0.513112	18.435	15.510	37.968
DR10-3	148.50	57.89	0.702470	0.513127	18.413	15.498	37.927
DR11-1g	146.80	57.63	0.702435	0.513125	18.401	15.494	37.862
DR11-3	146.80	57.63	0.702469	0.513129	18.404	15.499	38.072
DR12-1g	146.29	57.18	0.702310	0.513150	18.015	15.473	37.506
DR12-3g	146.29	57.18	0.702375	0.513146	18.202	15.481	37.690
DR12-3	146.29	57.18	0.702395	0.513170	18.204	15.485	37.699
DR13-2g	145.74	56.57	0.702556	0.513142	18.500	15.497	37.966
DR13-3	145.74	56.57	0.702570	0.513145	18.514	15.509	38.008
DR14-2	145.09	56.00	0.702538	0.513138	18.512	15.511	38.209
DR14-3	145.09	56.00	0.702530	0.513102	18.755	15.523	38.386
DR14-4	145.09	56.00	0.702557	0.513131	18.774	15.512	38.382

In sample name, g indicates glass.

* Isotopic composition normalized to $^{88}\text{Sr}/^{86}\text{Sr} = 0.1194$ and referred to NBS987 = 0.710250 ± 0.000015 .

† Isotopic composition normalized to $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$ and referred to La Jolla Nd = 0.511852 ± 0.000010 .

‡ Isotopic data were corrected for instrumental mass fractionation by applying a discrimination factor determined by multiple analyses of NBS981: all Pb isotopic ratios are relative to NBS981; $^{206}\text{Pb}/^{204}\text{Pb} = 16.9373$, $^{207}\text{Pb}/^{204}\text{Pb} = 15.4925$, $^{208}\text{Pb}/^{204}\text{Pb} = 36.7054$. Analytical uncertainties for Pb isotopic ratios are approximately ± 0.005 for $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{207}\text{Pb}/^{204}\text{Pb}$, and ± 0.01 for $^{208}\text{Pb}/^{204}\text{Pb}$. Total procedural blanks are <0.3 ng for Pb.

and the Darwin rise are the sites of most Pacific hotspots over the past 120 Myr (ref. 14) and correspond to a prominent, long-wavelength geoid high¹⁸ and to a zone of low seismic velocities extending to the lower mantle¹⁹. The Superswell may thus be located over a stable, persisting zone of upwelling from the lower mantle^{7,20} which may have been acting as a barrier since at least Cretaceous times, separating two large mantle domains with different convecting histories.

We discuss two interpretations of the above three observations. (1) The pollution hypothesis: the data can be interpreted in terms of the southern N-MORB being polluted by sources having a HIMU (that is, 'high μ ', where $\mu = {}^{238}\text{U}/{}^{204}\text{Pb}$) end component affinity (high ${}^{206}\text{Pb}/{}^{204}\text{Pb}$, low ${}^{87}\text{Sr}/{}^{86}\text{Sr}$), where the northern N-MORB would be polluted by sources having an EM (enriched mantle) end component affinity (high ${}^{87}\text{Sr}/{}^{86}\text{Sr}$). This view is supported by the observation that the 25°S boundary coincides with the SOPITA (Fig. 1), which has its northern part dominated by EM (Samoa, Societies and Marquesas) whereas its southern part is dominated by HIMU (Cook-Austral and Foundation chains)^{14,21,22}. An alternative view, based on the observation that the relative slopes are indistinguishable in Pb–Pb plots²³, would be that the northern province has experienced more pollution by EM sources on a time-integrated basis than the southern province. (2) Melting hypothesis: according to the 'plum-pudding' model²⁴, the MORB mantle source is composed of small-scale, uniformly distributed mantle heterogeneities which are expected to have high ${}^{87}\text{Sr}/{}^{86}\text{Sr}$ (relative to ${}^{143}\text{Nd}/{}^{144}\text{Nd}$ or ${}^{206}\text{Pb}/{}^{204}\text{Pb}$). These heterogeneities would be preferentially sampled^{25,26} by the low extents of melting which may prevail beneath the axis of the deeper, northern province. The higher extents of melting expected in the shallower, southern province would average the properties of mantle components, resulting in lower (${}^{87}\text{Sr}/{}^{86}\text{Sr}$)/(${}^{143}\text{Nd}/{}^{144}\text{Nd}$) and (${}^{87}\text{Sr}/{}^{86}\text{Sr}$)/(${}^{206}\text{Pb}/{}^{204}\text{Pb}$). However, the differences of mantle temperatures and melt parameters between the two provinces are not resolved by a petrological model²⁷ based on Na_8 data (Na normalized to 8% MgO).

These two hypotheses may be reconciled by considering the link between the chemical characteristics of the mantle and its physical properties (thermal state) as expressed at the surface by the bathymetry of the ridge. To summarize, the Pacific mantle displays at the same time large-scale variation of composition and temperature.

The rather sharp bathymetric²⁸ and geochemical²⁹ transition at 25°S indicates a superficial origin, whereas the cluster of hotspots constituting the Pacific Superswell suggests the existence of a deep-mantle process. This apparent paradox requires some interactions between the deeper and the shallower layers of the mantle. We propose that the lower-mantle upwelling suspected to be present near 25°S could have contributed to the isolation of two large-scale, deep-mantle domains with their own convective histories, producing slight differences in their chemical properties, thus influencing differently the thermal structure of the overlying MORB mantle source layer. □

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A therizinosauroid dinosaur with integumentary structures from China

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Therizinosauroida ('segnosaurs') are little-known group of Asian dinosaurs with an unusual combination of features that, until recently, obscured their evolutionary relationships. Suggested affinities include Ornithischia¹, Sauropodomorpha^{2,3}, Theropoda^{4–11} and Saurischia *sedis mutabilis*¹². Here we describe a new therizinosauroid from the Yixian Formation (Early Cretaceous, Liaoning, China)¹³. This new taxon provides fresh evidence that therizinosauroids are nested within the coelurosaurian theropods^{8–11}. Our analysis suggests that several specialized therizinosauroid characters, such as the Sauropodomorpha-like tetradactyl pes^{1,2}, evolved independently within this group. Most interestingly, this new dinosaur has integumentary filaments as in

Sinosauropteryx^{14,15}. This indicates that such feather-like structures may have a broad distribution among non-avian theropods, and supports the hypothesis that the filamentous integumentary structures may be homologous to the feathers of birds^{14,15}.

Dinosauria Owen 1842

Theropoda Marsh 1881

Coelurosauria *sensu* Gauthier 1986

Therizinosauroida Russell and Dong 1993

Beipiaosaurus inexpectus gen. et sp. nov.

Etymology. Beipiao: the city near the locality where the specimen was found; saurus: lizard; inexpectus: referring to the surprising features in this animal.

Holotype. IVPP V11559 (Institute of Vertebrate Paleontology & Paleoanthropology, Beijing, China; see Fig. 1).

Locality and horizon. Sihetun locality near Beipiao, Liaoning, China. The lower part of the Yixian Formation, probably from the Lower Cretaceous based on latest radiometric evidence¹³.

Diagnosis. *Beipiaosaurus inexpectus* differs from other therizinosauroids in having shorter and more bulbous tooth crowns, a larger skull, a tridactyl pes with a splint-like proximal first metatarsal, a shallow anterior iliac process, a long manus (10% longer than a femur), a long tibia (275 mm > 265 mm of the femur), an elongated lateral articular surface on the palmar side of manual phalanx I-1, and proximally compressed metatarsals III and IV.

Beipiaosaurus is the largest known theropod from the Yixian Formation, with an estimated length of 2.2 m. It has a relatively large skull compared to other therizinosauroids (preserved dentary is 65% of femur length). The anterior end of its dentary is downturned. The dentary has a lateral shelf, similar to other therizinosauroids and ornithischians¹. *Beipiaosaurus* has a large number of teeth (more than 37, inferred from the preserved alveoli in the broken dentary). They resemble those of *Protarchaeopteryx*¹⁶, but have larger serrations (3 serrations per mm) as in other therizinosauroids and troodontids⁹. Replacement teeth developed in oval resorption pits next to the roots of erupted teeth (Fig. 2a), as in *Archaeopteryx*¹⁷. Dorsally pointed, triangular interdental plates are present.

The cervical vertebrae bear low, anteroposteriorly short neural spines. Lateral depressions are present on the lateral sides of the centra of the fused posterior dorsals.

The coracoid is subrectangular, as in some maniraptoran theropods, with a pronounced coracoid tubercle. Exquisite impressions show that the furcula is a widely arched bone, oblate-shaped in cross section, without a hypocleidium. Compared to the short and stout hindlimb, the forelimbs are relatively long. The elongate hand is longer than the foot, as in dromaeosaurids and primitive *Avialae*¹⁸. As in other therizinosauroids, the humerus has a pointed internal tuberosity on its proximal end, and anteriorly positioned radial and ulnar condyles on its distal end. A depression on the proximal surface of the humerus separates the head and internal tuberosity, as in other therizinosauroids and *Mononykus*¹⁹. Five carpals are preserved. The largest distal carpal, the semilunate (Fig. 2c, d), is smaller than but otherwise identical to that of *Deinonychus*²⁰. It primarily contacts metacarpal II but also touches metacarpal I (Fig. 2d), unlike the condition in *Alxasaurus*, in which the largest carpal is the distal carpal I⁸. Distal carpal I is large and oval (Fig. 2c). The proximal carpals are represented by a V-shaped radiale in close contact with the radius, and a small rounded carpal between the distal ends of the radius and ulna (Fig. 2c, d). The manus is slender and elongate, proportionally similar to that of *Deinonychus*²⁰. Metacarpal I has a pronounced distal flange, as in *Deinonychus*. The proximal parts of metacarpals I and II are closely appressed. Metacarpal III is slender and slightly bowed. The combined lengths of phalanges III-1 and III-2 are equal to the length of phalanx III-3, as in advanced theropods². There are well developed ligament pits on the lateral sides of the distal ends of the phalanges. The manual unguals are laterally compressed and strongly curved. As in other

therizinosauroids⁸, their proximal ends are deep but taper to needle-sharp points. The second manual claw is slightly longer than the first, resembling those of *Archaeopteryx* and *Protarchaeopteryx*²¹.

The ilium is shaped like a parallelogram, similar to those of dromaeosaurids and basal birds, but unlike the sauropod-like ilia of derived therizinosauroids^{1,22}. The posterodorsal margin of the ilium curves ventrally in lateral view. The anterior and posterior processes are almost the same length. The posteroventral margin of the ilium is deflected laterally at a right angle to the vertical ramus, and has a shallow brevis fossa similar to those of other coelurosaurians²³. The partial pubic peduncle of the ilium is longer than the ischiadic peduncle, similar to those of therizinosauroids, dromaeosaurids and *Archaeopteryx*²³. Both the pubic and the ischial shafts are more rounded than flattened, unlike those of *Alxasaurus* and *Segnosaurus*. As in some theropods, the pubic apron is compressed and positioned more distally. The femur of *Beipiaosaurus* has a wing-like lesser trochanter, a cleft between the greater trochanter and the lesser trochanter, and a crest-like fourth trochanter. The tibia has a fibular crest, a feature of theropods². The fibula is very slender compared to the tibia, especially the distal half. As in *Alxasaurus*⁸ and the *Avialae*²⁴, the medial surface of the fibula is flat, lacking the medial fossa of some theropods. As in other therizinosauroids, the astragalus has a tall ascending process and reduced condyles that only partly cover the distal end of the tibia. The calcaneum is sub-oval and disk-shaped. The metatarsus is 39% of the length of the tibia, larger than in known therizinosauroids but less than in other theropods (>45%)⁸. The proximal end of metatarsal I is flattened and tapered and, as in most maniraptorans, does not contact the tarsus (Fig. 2e, f). The proximal ends of both metatarsals III and IV are compressed, especially on the medial side. Metatarsal V is slender and strap-like, being only half the length of the other metatarsals. One pedal ungual is preserved, and is shorter than any manual unguals.

Large patches of integumentary structures were found in close association with the ulna, radius, femur and tibia, as well as with pectoral elements. The filamentous structures are best preserved near the ulna, almost perpendicular to the bone (Fig. 3). They are similar to the integumentary structures of *Sinosauropteryx*¹⁵ in their parallel arrangement. Unlike those of *Sinosauropteryx*, the integumentary structures of *Beipiaosaurus* contact the ulna. They are densest close to the bone. Most of the integumentary filaments are about 50 mm long, although the longest is up to 70 mm. Some filaments have shallow and faint median grooves, possibly indicating hollow cores that had collapsed, and have indications of branching distal ends as in *Sinosauropteryx*¹⁵. As in *Sinosauropteryx*¹⁵ and birds from the same locality, it is difficult to isolate a single filament and thus difficult to describe the branching pattern of the integumentary filaments.

Therizinosauroida has many perplexing features for a theropod, such as a very small head, a sauropod-like ilium and a short and broad tetradactyl pes with rudimentary metatarsal V^{1,2,12,22}. Until now, no cladogram has been proposed for the relationships and morphological evolution of therizinosauroids. We ran a phylogenetic analysis with an 84-character dataset (see Supplementary Information for the character list and matrix). We left out the unnamed 'segnosaur' from the Early Jurassic Lower Lufeng Formation²⁵ as it is too incomplete. Using PAUP (3.1.1. Exhaustive search, Deltran optimization; Swofford, 1993), we obtained a single most parsimonious tree (tree length, 133; consistency index, 0.707; retention index, 0.645; rescaled consistency index, 0.456). Our analysis (Fig. 4) places *Beipiaosaurus* as a basal taxon within Therizinosauroida. *Beipiaosaurus* has a relatively large skull (1.0) among therizinosauroids, a tridactyl pes (79.0) and a fibular crest on the tibia, all of which are primitive theropod features. The pelvic elements are also very similar to those of other coelurosaurians. These characteristics support the hypothesis that therizinosauroids (including *Beipiaosaurus*) are nested within the coelurosaurian

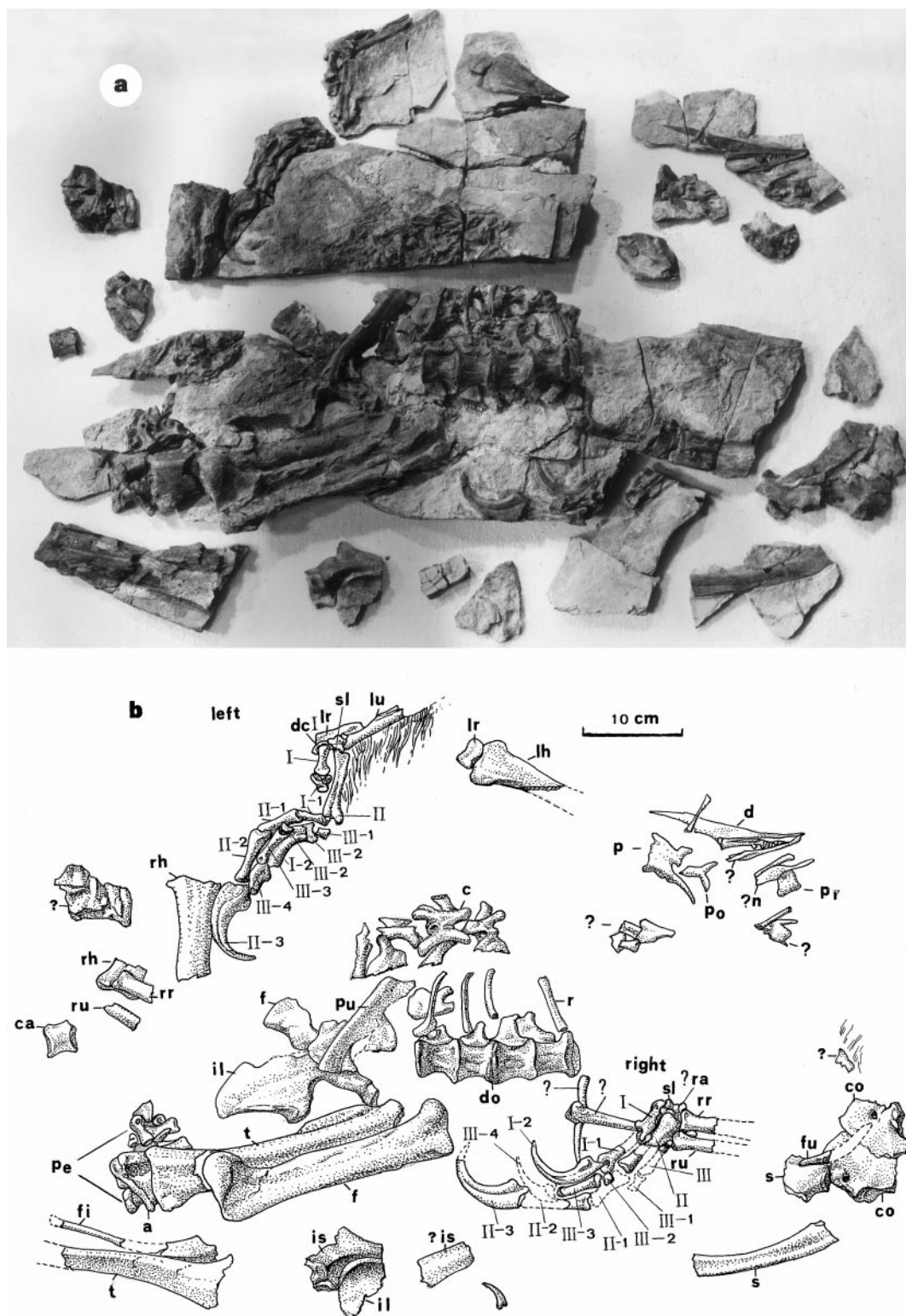


Figure 1 *Beipiaosaurus inexpectus* (V11559, holotype). Photograph (a) and outline (b) of the skeleton (broken lines indicate features preserved in impressions). The holotype was collected in 1996 by a farmer, Li Yinxian, from the famous Sihetun locality. It was later (1997) determined to be from the lower part of the Yixian Formation. According to communication with the collector, and consistent with the close proximity, preservation and proportions of the elements, all elements (including the integumentary structures) are from a single individual. V11559 includes the partial right dentary with dentition, right postorbital, right parietal, right nasal?, right prootic, a few cervicals and dorsals, an incomplete caudal, incomplete ribs, partial scapula, coracoids and furcula, partial humerus,

radius and ulna, nearly complete hands, partial ilium, pubis and ischium, complete right femur, right tibia and right fibula, incomplete left femur, tibia and fibula, incomplete right foot. Some elements are represented by impressions. Sacral and most caudal vertebrae are missing. a, astragalus; c, cervical vertebra; ca, caudal vertebra; co, coracoids; d, dentary; dcl, distal carpal I; do, dorsal vertebra; f, femur; fi, fibula; fu, furcula; I–III, metacarpals I–III, I–1 to III–4, manual phalanges I–1 to phalanges III–4; il, ilium; is, ischium; lh, left humerus; lr, left radius; lu, left ulna; ?n, ?nasal; p, parietal; pe, pes; po, postorbital; pr, prootic; pu, pubis; r, rib; ?ra, ?radiale; rh, right humerus; rr, right radius; ru, right ulna; s, scapula; sl, semilunate distal carpal; t, tibia.

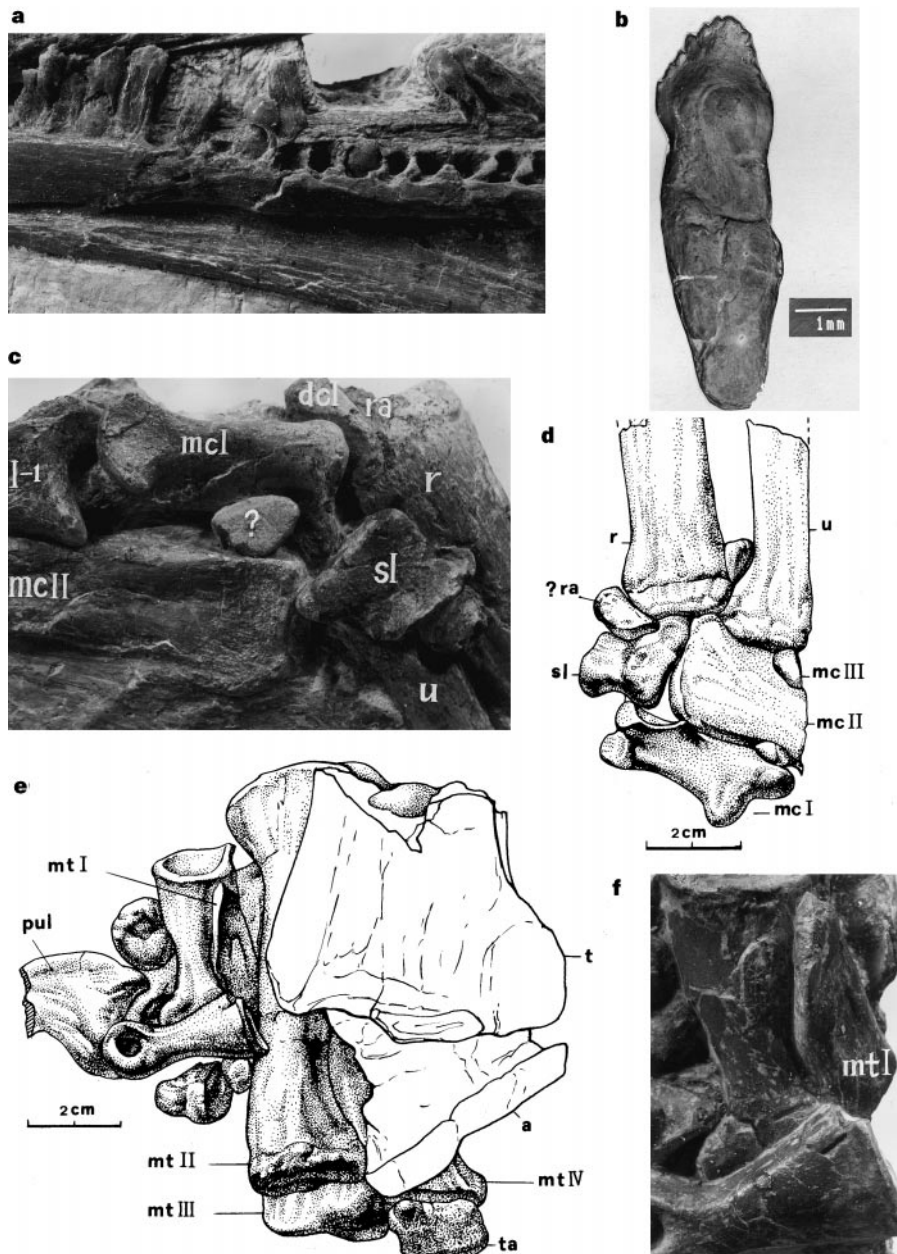


Figure 2 *Beipiaosaurus inexpectatus*. **a**, Nine right dentary teeth in medial view. Note the resorption pits and replacement teeth. **b**, A dentary tooth in lateral view. **c**, Close-up of the left semilunate carpal of V11559. **d**, Drawing of part of the right manus of V11559. Note the shape and position of the semilunate, which is very similar to that of birds¹⁷. **e**, Drawing of the partially preserved right pes of V11559.

f, Close-up of the first metatarsal of V11559. Note the proximally pinched theropod first metatarsal. The theropod first metatarsal is absent in other therizinosauroids, which has been argued as being strong evidence against the theropod affinities of therizinosauroids¹. Additional abbreviations: mc I–III, metacarpals I–III; mt I–IV, metatarsals I–IV; pul, pedal ungual; r, radius; ra, radiale; ta, tarsal; u, ulna.

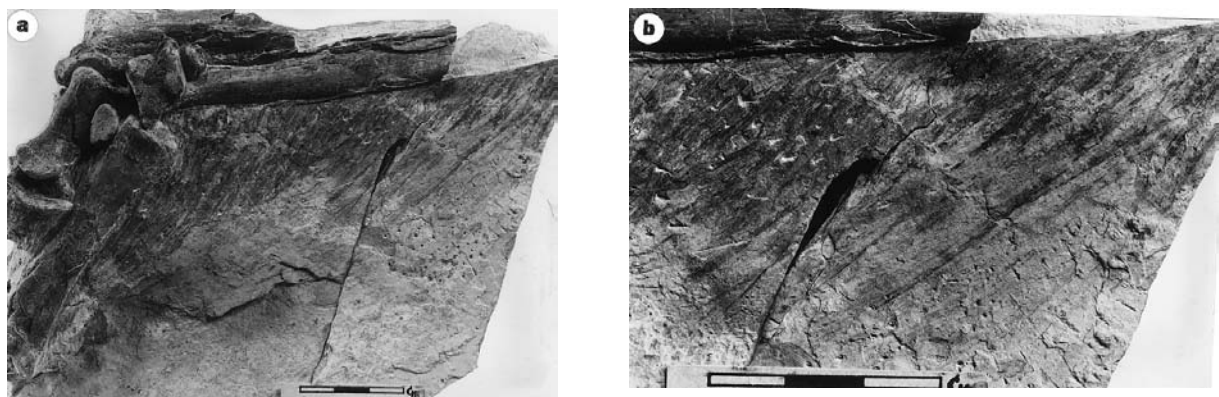


Figure 3 *Beipiaosaurus inexpectatus*. **a**, Partially preserved forelimb with unusual integumentary impression. **b**, Close-up of the integumentary impression.

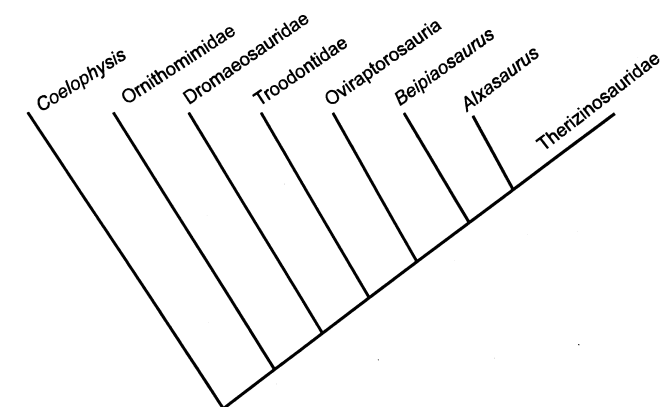


Figure 4 Phylogenetic relationships of *Beipiaosaurus inexpectus*. *Beipiaosaurus* and other therizinosaurids share 18 synapomorphies, including the following unique characters: a prominent dorsolateral shelf on the dentary (21.1), teeth that increase in size anteriorly (25.1), tooth crowns with sub-circular basal cross-sections that lack mediolateral compression (27.1), anteroposteriorly narrow and dorsoventrally deep pubic peduncle of ilium (46.1 and 47.1), very deep proximal end of manual unguals (70.1), short metatarsus (78.1) and reduced main body of astragalus (82.1). It is less derived than other therizinosaurids because it lacks 13 characters of Therizinosauridae (1.1, 36.1, 38.0, 43.0, 48.1, 49.1, 51.1, 52.1, 58.1, 60.0, 66.0, 77.1, 79.1), including the following unusual characters: a very small head (1.1), the long and deep preacetabular portion of ilium (48.1 and 49.1) and absence of the theropod first metatarsal (79.1).

theropods^{8–11}. Given this phylogeny (Fig. 4), some derived characters of therizinosaurids other than *Beipiaosaurus* are most parsimoniously interpreted as having evolved convergently with some other dinosaur groups, sauropodomorphs in particular. Thus, therizinosaurids re-evolved a robust first digit in which the proximal end of metatarsal I articulates with the tarsals (79.1).

Feathers are complex structures. Their abrupt appearance in the bird fossil record has been difficult to explain, mainly because no intermediate structures are preserved in the related theropod taxa. The integumentary filaments of *Sinosauropteryx* have been considered to be 'proto-feathers' by some, but this idea has been rejected by others²⁶. Such structures have not been preserved with any other theropods²⁶ until the discovery of *Beipiaosaurus*. The filamentous structures in *Beipiaosaurus* are similar to, but longer than, those of the compsognathid *Sinosauropteryx*. They are perpendicular to the limb bones, and are unlikely to be muscle fibres or frayed collagen²⁷. Their presence in both therizinosaurids and compsognathids indicates that there may be a broader distribution of similar structures in theropod dinosaurs. This supports the idea that these simple integumentary filaments may represent an intermediate evolutionary stage to the more complex feathers of *Protarchaeopteryx*, *Caudipteryx*¹⁶ and more derived Avialae. The absence of such structures in most theropod fossils is probably attributable to the lack of such ideal preservation as is found in the Yixian Formation. This again indicates that feathers preceded flight¹⁶, because both therizinosaurids and compsognathids apparently could not fly and did not descend from flying animals. □

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Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

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Complex dynamics and phase synchronization in spatially extended ecological systems

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Population cycles that persist in time and are synchronized over space pervade ecological systems, but their underlying causes remain a long-standing enigma^{1–11}. Here we examine the synchronization of complex population oscillations in networks of model communities and in natural systems, where phenomena such as unusual '4- and 10-year cycle' of wildlife are often found. In the proposed spatial model, each local patch sustains a three-level trophic system composed of interacting predators, consumers and vegetation. Populations oscillate regularly and periodically in phase, but with irregular and chaotic peaks together in abundance—twin realistic features that are not found in standard ecological models. In a spatial lattice of patches, only small amounts of local migration are required to induce broad-scale 'phase synchronization'^{12,13}, with all populations in the lattice phase-locking to the same collective rhythm. Peak population abundances, however, remain chaotic and largely uncorrelated. Although synchronization is often perceived as being detrimental

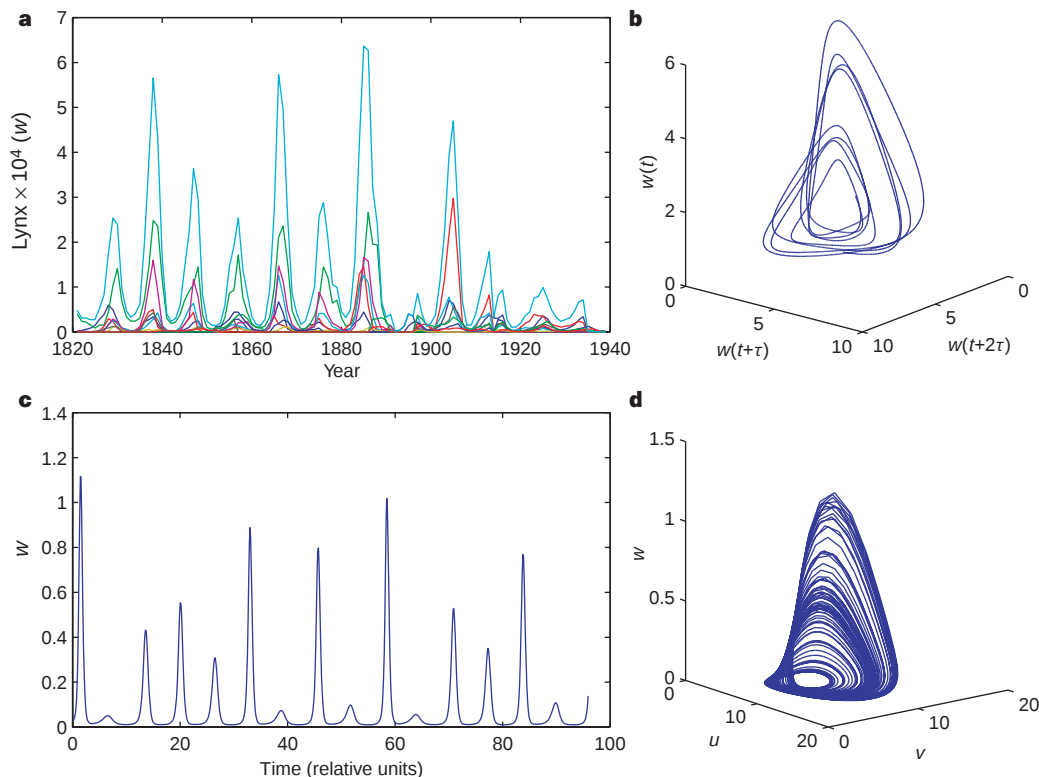


Figure 1 Time series analysis. **a**, Time series of lynx abundances (1821–1934) from six regions in Canada. Data are from ref. 1. **b**, Reconstructed attractor of hare-lynx system obtained by spatially averaging all regional lynx data and embedding the resulting time-series $w(t)$ using lagged coordinates, $w(t)$ versus $w(t+3)$ versus $w(t+6)$ after filtering and interpolating¹¹. The dynamics of the full three-dimensional system is reconstructed from the time series of the single lynx

variable. The attractor ‘folds’ in three-dimensional space, showing the chaotic cycling between predator, prey and vegetation. **c**, Time series of model (equation (1)) predator population w with chaotic dynamics. **d**, The model’s hare-lynx-vegetation attractor, obtained by plotting u versus v versus w , has a similar structure to that found in **a**.

to spatially structured populations¹⁴, phase synchronization leads to the emergence of complex chaotic travelling-wave structures which may be crucial for species persistence.

Ecological systems and their component biological populations exhibit a broad range of non-equilibrium dynamics, from characteristic natural cycles to more complex chaotic oscillations^{15–17}. Here we examine a common but intriguing class of ecological cycle in

which the frequency of a population remains relatively constant over time but in which there are erratic changes in abundance. A striking example of this is the well known hare-lynx cycle^{1–11}. Despite unpredictable population fluctuations from one cycle to the next in the snowshoe hare (*Lepus americanus*) and the Canadian lynx (*Lynx canadensis*), the overall oscillation tends to follow a tight rhythm with a period of ~10 years¹¹ (Fig. 1a). Hare and lynx

Box 1 The chaotic UPCA foodweb model

We used the following vertical foodweb model with vegetation (u), herbivores (v) and predators (w):

$$\begin{aligned}\dot{u} &= au - \alpha_1 f_1(u, v) \\ \dot{v} &= -bv + \alpha_1 f_1(u, v) - \alpha_2 f_2(v, w) \\ \dot{w} &= -c(w - w^*) + \alpha_2 f_2(v, w)\end{aligned}\quad (1)$$

Parameters a , b and c represent the respective growth rates of each trophic species in the absence of interspecific interactions. Predator-prey and consumer-resource interactions are incorporated into the equations using either the Lotka-Volterra term, $f_i(x, y) = xy$, or the Holling type II term, $f_i(x, y) = xy/(1 + k_i x)$, with strengths set by the coefficients α_i . We also generalize the model by allowing the predator w to maintain a low equilibrium level $w = w^*$ even when its usual prey, v , is rare. This might arise when there are alternative food sources available for the predator²², and is modelled by linearizing the predators’ growth rate in equation (1) about the equilibrium by using the term $(w - w^*)$. These equations might, for example, outline the principal ecological transfers involved in the Canadian lynx-hare-vegetation foodweb, whose dynamics are dependent on three vertical trophic levels^{3,28}

and where alternative prey (such as the red squirrel) are considered to be important for the lynx population²⁹.

For the simulation runs reported here, we used the following parameters: $a = 1$, $b = 1$, $c = 10$, $\alpha_1 = 0.2$, $\alpha_2 = 1$, $k_1 = 0.05$, $k_2 = 0$, $w^* = 0.006$. f_1 and f_2 were taken as Holling type II and Lotka-Volterra interaction terms, respectively, although different combinations have been used successfully in other model variants. Chaotic dynamics were diagnosed from a study of the model’s Lyapunov exponent, calculated as in ref. 30.

In an N -patch system, migration to the i th patch is modelled as follows:

$$\begin{aligned}\dot{u}_i &= au_i - \alpha_1 f_1(u_i, v_i) \\ \dot{v}_i &= -bv_i + \alpha_1 f_1(u_i, v_i) - \alpha_2 f_2(v_i, w_i) + D \sum_j (v_j - v_i) \\ \dot{w}_i &= -c(w_i - w^*) + \alpha_2 f_2(v_i, w_i) + D \sum_j (w_j - w_i)\end{aligned}\quad (2)$$

w_i represents the predator population in the i th patch and D sets the magnitude of diffusive migration summed over a predefined set of local nearest neighbours $\{j\}$. Lattice simulations (as in Fig. 5), were obtained for free and periodic boundaries in $N \times N$ lattices for N values of 20, 50 and 100, and with coupling through 4 and 8 nearest neighbours. Results were robust to these changes in model configuration.

populations from different regions of Canada synchronize in phase to a collective cycle that manifests over millions of square kilometres^{1–10}. Similar spatially synchronized fluctuations are seen in many biological, ecological and epidemiological contexts and may involve disparate animal taxa across widely separated sites^{7–10,18}.

There are many possible explanations for spatially synchronized population oscillations. It has been suggested that extrinsic large-scale climatic forcing may often be responsible for entraining populations over vast geographic distances⁷. Intrinsic predator–prey and consumer–resource relationships within the foodweb, including density-dependent and time-delayed effects, may also generate population cycling, with local migration enhancing spatial synchronization^{6,9,10}. The large number of ecological processes involved underscores the need to develop a deterministic ‘strategic model’¹² that can realistically reproduce these complex population dynamics. A simply formulated robust tritrophic foodweb model achieves this goal and provides the necessary framework for studying ecological synchronization effects. The minimal model consists

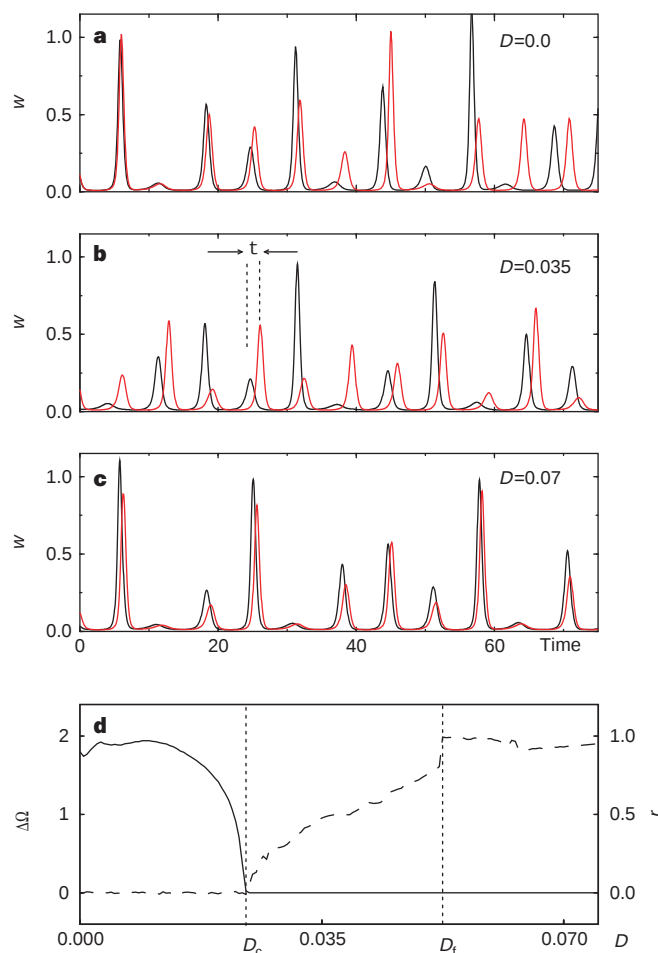


Figure 2 Model time series of predator populations in a two-patch system ($b_1 = 1.1$, $b_2 = 1.055$) under different synchronization regimes. **a**, $D = 0.0 < D_c$ (unsynchronized); **b**, $D = 0.035 > D_c$ (phase synchronized); **c**, $D = 0.07 > D_f$ (full synchronization). **d**, Population time series were decomposed into time-varying phase $\phi(t)$ and amplitude $A(t)$ components (Box 2). For the two-patch system, the relative frequencies, $\Delta\Omega = 100 \times (\Omega_2 - \Omega_1)/\Omega_1$, per cent (solid line), determined using equation (3), together with the correlation r between peak population abundances A_1 and A_2 (dashed line), are plotted as a function of coupling D . Three different regimes are seen: $D < D_c$ (left)–the patches are unsynchronized ($\Delta\Omega > 0$) and slowly drifting in and out of phase, with correlation between peak population abundances $r = 0$; $D_c < D < D_f$ (middle)–the patches are ‘phase synchronized’ ($\Delta\Omega = 0$), with peak population abundances chaotic but weakly correlated $r \ll 1$; $D > D_f$ (right)–the patches are almost fully synchronized ($\Delta\Omega = 0$), with nearly identical populations and $r \approx 1$.

of a three-level ‘vertical’ food chain with predators (w) feeding on herbivores (v), which consume vegetation (u), and is described in Box 1.

This novel skeleton model generates surprisingly complex dynamics, including equilibrium and limit-cycle behaviour, and large parameter ranges for which there is an interesting class of chaotic oscillation. Figure 1c shows a time series of the predator population (w) for a typical model run in the chaotic regime. The predator, like all model populations, oscillates with a frequency Ω that is practically constant¹², as the community trajectory rotates at a uniform phase rate around the ‘attractor’ (Fig. 1d). Despite this regular rhythm in phase, the peak population abundance in each cycle appears to be highly unpredictable. These twin features of uniform phase evolution and chaotic amplitude (UPCA) are similar to those of the Canadian hare–lynx system¹¹, as is evident from comparing their respective time series and underlying ‘attractors’ (Fig. 1). The model lynx population achieves a cycle-amplitude variability (ratio of maximum to minimum population density) of 12–150, which is very close to recorded field values⁹. Although other deterministic tritrophic models also yield chaotic oscillations^{4,19–21}, none reproduces both the regular recurring rhythm and the smooth but irregular rises and falls in population abundances (the UPCA) fundamental to equation (1) and the observed hare–lynx data.

The population model (Box 1, equation (1)) represents a single isolated ‘patch’ or community. We add a spatial structure by analysing a set of tritrophic UPCA patch models interconnected by diffusive migration of strength D to form what might constitute a ‘metacommunity’²² (Box 1, equation (2)). We first examine the mutual interaction of two different patches (u_1, v_1, w_1) and (u_2, v_2, w_2) that show UPCA which, in the absence of migration ($D = 0$), would normally be unsynchronized. As the natural frequency Ω_i ($i = 1, 2$) of each uncoupled chaotic community is approximately a linear function of herbivore growth b_i , in our scheme different communities are specified by differences in b_i . Thus,

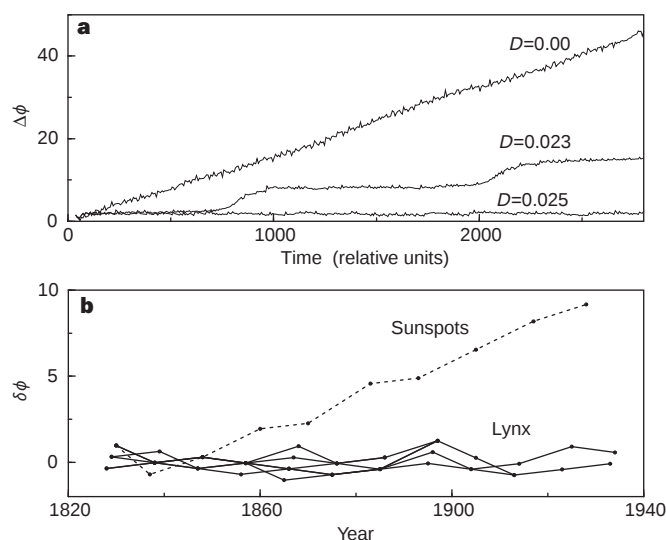


Figure 3 Phase analysis. **a**, Phase growth in two coupled patches. $D = 0$: unsynchronized with linear growth in phase difference between the two patches: $\Delta\phi(t) = \phi_1(t) - \phi_2(t)$, as described by equation (3) (Box 2); $D = 0.025 = D_c$: phase synchronized with constant inter-patch phase difference, $\Delta\phi(t) = \text{const}$; $D = 0.023 \leq D_c$: just before the onset of phase synchronization ($D \leq D_c = 0.025$), $\Delta\phi$ is generally constant, but sporadically jumps in value as ‘phase slips’ occur¹². **b**, Phase growth of the lynx data (from Fig. 1a). For each year, the mean phase growth $\bar{\phi}(t) = \langle \phi_i(t) \rangle$ is calculated as an average over all six regions, $i = 1.6$. For each region, the deviation of the phase growth from the mean ($\delta\phi_i(t) = \phi_i(t) - \bar{\phi}(t)$) is plotted. The phase growth of the filtered sunspot data, $\phi_s(t)$, in the same years (1821–1934) is compared to the mean lynx phase growth, $\bar{\phi}(t)$, by plotting $\delta\phi_s(t) = \bar{\phi}(t) - \phi_s(t)$ (Box 2).

$\Delta\Omega = \Omega_1 - \Omega_2 \approx b_1 - b_2$ is the frequency difference between two uncoupled unsynchronized communities.

Might these chaotic foodweb systems mutually synchronize after coupling and, if so, how? Although much is known about the synchronization of periodic oscillators, chaotic systems are less well understood¹². Consider the two-patch UPCA model (Box 1, equation (2)) under conditions of extremely weak coupling. When migration between patches is less than a numerically determined critical value ($D < D_c = 0.025$), the two patches retain their own intrinsic chaotic dynamics, and their populations remain completely independent and unsynchronized, both in amplitude and phase (Fig. 2a). It can be seen and quantitatively shown (Fig. 3a; Box 2) that the two communities cycle at different natural frequencies. In contrast, relatively strong coupling (above a numerically determined threshold; $D > D_c = 0.053$) induces a state of 'complete' or 'full' synchronization²³. Here, comparable populations in different patches are identical (that is, $u_1(t) = u_2(t)$, $v_1(t) = v_2(t)$, $w_1(t) = w_2(t)$), or very close to identical, even though the dynamics remain chaotic in time (Fig. 2c). Now the two communities entrain one another and cycle chaotically at the same frequency.

A more unusual form of synchronization emerges at weak or intermediate coupling levels ($D_c < D < D_f$). In this case, the predator populations (w_i) in each patch remain synchronized or locked in phase, but their peak population abundances appear to be

Box 2 Analysing phase dynamics in model and natural time series

We devised a practical method to identify subtle forms of synchronization in irregular or chaotic population time series. A cyclic variable $x(t) = A(t) \sin(\phi(t))$ may be decomposed into a time-dependent amplitude $A(t)$ and phase $\phi(t)$, although the decomposition is non-trivial for a chaotic signal^{12,13}. We calculate growth in phase ϕ in a time-varying (possibly chaotic) signal $x(t)$ simply by noting that the time between two successive peaks or maxima of $x(t)$ corresponds to an increase of 2π . Hence the instantaneous phase $\phi(t)$ at time t may be determined through linear interpolation after calculating the local rates at which maxima occur in the population time series. The amplitude $A(t)$ of the signal is defined here as the peak population abundance measured stroboscopically as each maximum occurs.

This decomposition technique can be used for detecting phase synchronization in two different population time series, with phases $\phi_1(t)$ and $\phi_2(t)$, respectively. Synchronization may be observed by monitoring the growth in time of the phase difference $\Delta\phi(t) = \phi_1(t) - \phi_2(t)$. When synchronized, $\Delta\phi(t)$ is, on average, constant in time and the two populations lock to the same frequency (frequency difference $\Delta\Omega = 0$). The patches are unsynchronized ($\Delta\Omega > 0$) when the average phase difference $\Delta\phi(t)$ grows with time according to:

$$\Delta\phi(t) = \Delta\Omega t + \tau \quad (3)$$

where the time-lag τ acts as a constant offset.

Monitoring phase evolution has many practical applications. For example, it provides an alternative test for checking whether the sunspot cycle entrains the hare-lynx populations^{5,25,26}. We analysed the phase growth of the Canadian lynx data over six geographically disparate regions (Fig. 1) and found it to be significantly faster than the phase growth of the sunspot cycle ($P < 0.01$; Fig. 3b). The average period lengths of the lynx and sunspot cycles were determined by regression as $T_l = 9.5 \pm 0.04$ (s.e.) and $T_s = 11.14 \pm 0.015$ (s.e.) years, respectively. Hence, in the long term, sunspot activity is unlikely to be involved in the continual entrainment of the hare-lynx cycle^{6,7}. On the other hand, the patterns in phase evolution between the six lynx populations were not significantly different, indicating phase synchronization over all six regions. In addition, the correlations between peak population abundances examined over all pairs of lynx populations were weak, with average $\langle r \rangle = 0.3$, making this synchronization akin to the phase synchronization observed in the model runs above.

independent, having very weak correlation (Fig. 2b). This phase synchronization implies that populations may be synchronized only in phase but not in amplitude¹². Because of its subtle nature, phase synchronization has been overlooked in population studies before now, but it may be very important because it occurs in the biologically realistic situation of weak coupling. The foodweb model provides one illustration of phase synchronization that closely matches the observed long-term dynamics of lynx populations (Fig. 1a). Although they are synchronized in phase, the populations of each patch nevertheless differ by a theoretically predicted²⁴ time lag (τ), the implications of which are discussed below. A summary of all synchronization regimes of the foodweb model is given in Fig. 2d.

Various realistic extensions of the foodweb model were analysed to check the robustness of our results. For example, when the system was simulated with hares and lynx migrating at different speeds, phase synchronization was still achieved over wide parameter ranges (see Supplementary Information). Given that stochasticity is ubiquitous in natural ecologies, we examined the response of the model to different types of noise forcing. When the parameters of the model were selected to give a regular periodic cycle and it

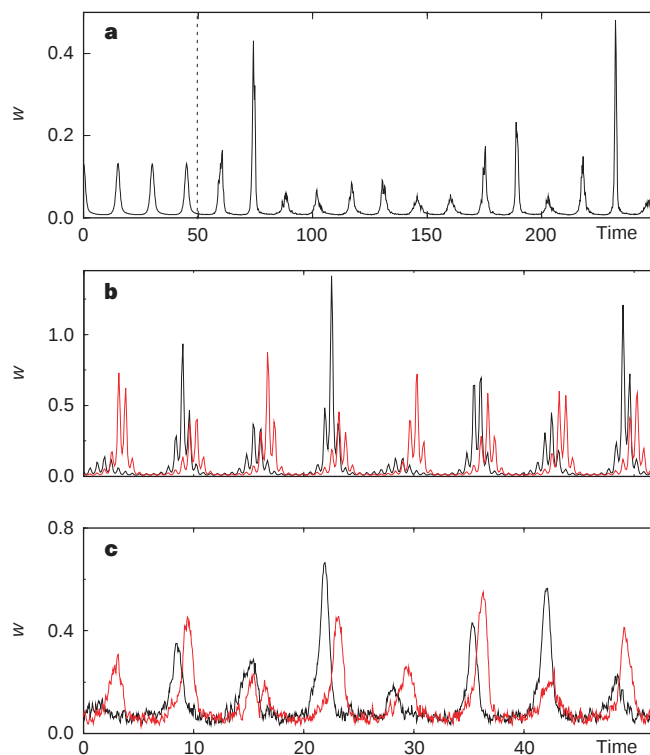


Figure 4 Effects of noise and seasonality. **a**, Noise-induced chaos. When the foodweb model is parametrized according to Box 1, but with $b = 0.2$, a limit-cycle solution results. At $t = 50$, environmental noise was added to the parameters a, b, c in equation (1) with, for example, $a'(t) = a(1 + e(t))$, where $e(t) = N(0, 0.05)$, and so on. The time series exhibited noise-induced chaos with the same UPCA dynamics as the deterministic model (equation (1)) (Fig. 1c). **b**, Robustness of phase synchronization to seasonal forcing. Seasonal forcing was added to the growth rates a, b, c in equation (1) with, for example, $a'(t) = a(1 + 0.1 \sin(w_c t))$ for the two patch model parametrized as in Fig. 2b. The frequency, w_c , of the forcing was chosen so that the intrinsic population cycle of the foodweb comprised ten seasonal cycles. The resulting population dynamics realistically portray seasonal dynamics, and the lynx populations (w) of the two-patch system remain in phase synchronization. **c**, Robustness of phase synchronization to noise. Demographic noise was added to all populations in the two-patch chaotic model (by adding noise to the right-hand side of all equations in equation (1)) parametrized as in Fig. 2b. The noise has mean zero and $\sigma = 0.08$ but is restricted so that at any instant the sum of the noise perturbation and the population level remains positive. Despite the noise forcing, the populations remain in phase synchronization.

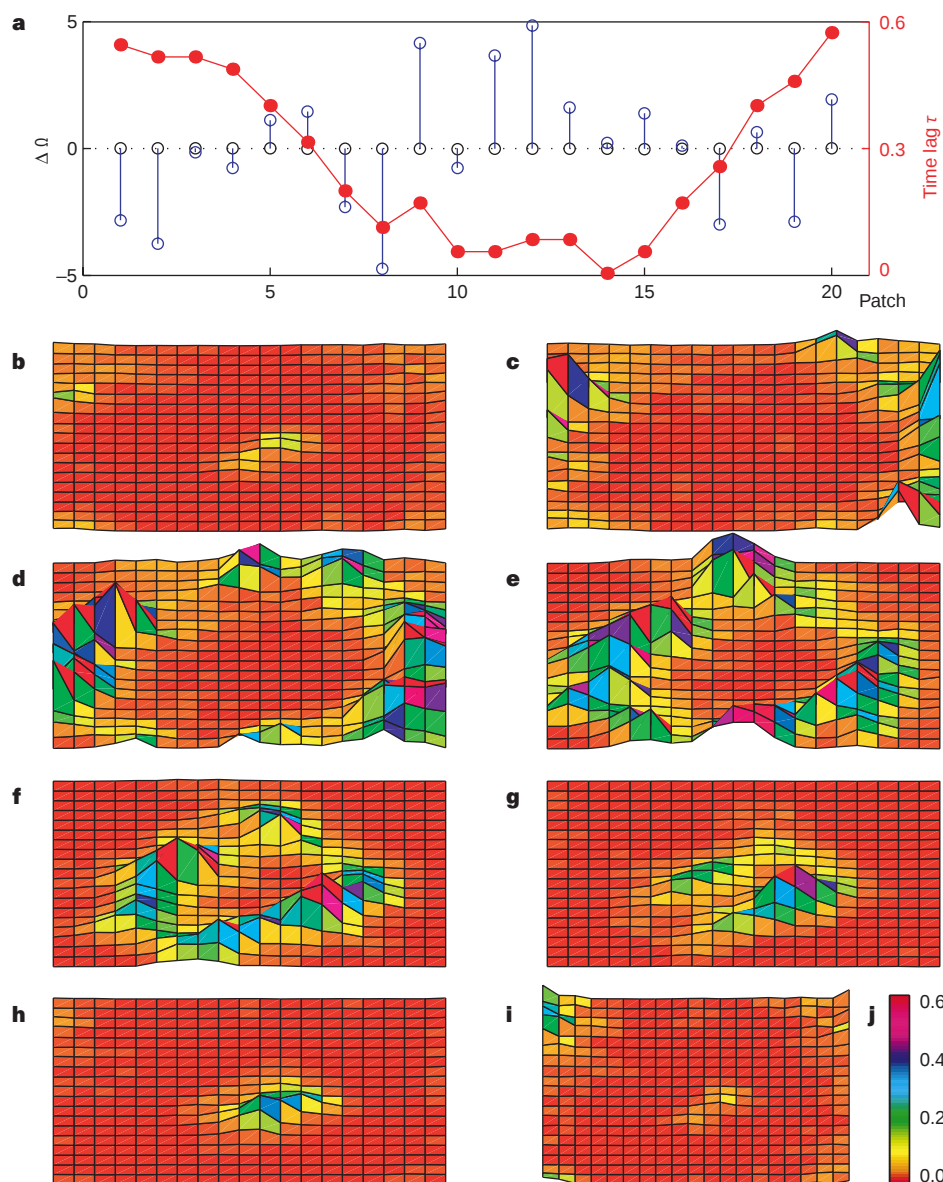


Figure 5 Simulation results in a square lattice of 20×20 sites with periodic boundary conditions (Box 1). **a**, For the 20 sites along the lattice diagonal with mean frequency $\bar{\Omega}$, we define the relative frequency of each patch as $\Delta\Omega_i = 100 \times (\Omega_i - \bar{\Omega})/\bar{\Omega}$. The relative frequencies $\Delta\Omega_i$ along the diagonal are plotted for $D = 0$ (blue) and $D = 0.035$ (black). Superimposed is a plot of τ_i (red), the time lag between the maximum of the predator abundance in the i th patch and the maximum of a central reference patch, for the 20 diagonal patches

determined when $D = 0.035$ (phase synchronization). Hence τ_i is measured as a fraction of the period length $T = 2\pi/\bar{\Omega}$. The distribution of the τ_i confirms the characteristic 'U' shape found in analyses of the field data^{10,25–27}. **b–i**, Evolution of a chaotic travelling wave: snapshots of predator abundance in the lattice at 8 consecutive time steps over one period ($D = 0.035$). The wave pattern in **b–i** repeats in an endless cycle, with patches having chaotic amplitudes, making each cycle different from the next. **j**, Colour bar shows abundance levels.

was forced with environmental or demographic stochasticity, an unusual form of noise-induced chaos resulted (Fig. 4a). The population dynamics exhibited the same UPCA oscillations as the purely deterministic model (Fig. 1c). In the coupled two-patch foodweb model, neither short-term noisy fluctuations (such as measurement, demographic or environmental noise) nor long-term seasonal changes interfered with the transition to phase synchronization (Fig. 4b, c). In all cases that showed phase synchronization, the actual transition to synchronization was rapid and emerged after only two or three population cycles. Synchronization transitions were found to be complex functions of parameter values (see Supplementary Information).

Finally, we constructed a spatial lattice of $N \times N$ non-identical patch foodwebs (where N was 20, 50 or 100), with each patch consisting of a single tritrophic model (equation (1)) linked by migration to its eight nearest neighbours. All patches were given

random consumer growth rates, b_i , uniformly distributed to lie within $\pm 10\%$ of the mean $\langle b_i \rangle = 1$. As expected, with no migration between patches ($D = 0$), the populations displayed independent and unsynchronized chaotic oscillations which varied by $\pm 5\%$ from the mean frequency (Fig. 5a). However, with very low migration ($D = 0.035$), the entire lattice became phase synchronized to a common coherent frequency (Fig. 5a). Despite the strong phase-locking, the 'amplitudes' of patch populations were only weakly correlated ($\langle r \rangle = 0.2$ between all patch pairs).

Note that if all patches were in 'full synchronization', their phase and amplitude dynamics would be identical and populations across the entire lattice would increase and decrease simultaneously. However, when they are phase-synchronized, inter-patch populations peak almost, but not quite, concurrently, with the peaks of pairs of populations being temporally separated by a time lag (as in Fig. 2b). When viewed over a large metacommunity, the time lags

show a characteristic 'U' shape (Fig. 5a). This gives rise to a remarkable chaotic travelling-wave structure which may be seen in the eight lattice 'snapshots' of Fig. 5b–i. Population abundances in the meta-community remain chaotic, but periodic circular waves continuously expand and contract radially as they spread in time across the spatial landscape. Field and model population studies of the Canadian hare–lynx cycle and European vole cycles have found similar travelling-wave structures with spatially distributed U-shaped phase lags^{10,25–27}. Different types of realistic diffusion barrier were introduced into the model but, in general, they failed to destroy the spatial circular wave structure (B.B. *et al.*, manuscript in preparation).

The spatio-temporal structures associated with phase synchronization have important implications for conservation ecology. Even if a disturbance perturbs a local patch population to the brink of extinction, the periodicity of spatial phase synchronization guarantees the recurring arrival of wave fronts in which new colonizers will buffer the endangered population. In contrast to the common view of population synchronization as a cause of global population extinctions¹⁴, it appears that phase synchronization can be important for maintaining species persistence. Our findings indicate that synchronization is a powerful process that has the potential to shape the distribution and abundance of species over all scales, from local to continental. We expect that the complex synchronization phenomena identified here will provide new insight into the dynamics of spatial ecologies and will have important applications to the study of biological rhythms in general. □

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Auditory collusion and a coupled couple of outer hair cells

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The discrepancies between measured frequency responses of the basilar membrane in the inner ear and the frequency tuning found in psychophysical experiments led to Békésy's idea of lateral inhibition in the auditory nervous system¹. We now know that basilar membrane tuning can account for neural tuning², and that sharpening of the passive travelling wave depends on the mechanical activity of outer hair cells (OHCs)³, but the mechanism by which OHCs enhance tuning remains unclear. OHCs generate voltage-dependent length changes at acoustic rates^{4–8}, which deform the cochlear partition^{9–11}. Here we use an electrical correlate of OHC mechanical activity, the motility-related gating current, to investigate mechano-electrical interactions among adjacent OHCs. We show that the motility caused by voltage stimulation of one cell in a group evokes gating currents in adjacent OHCs. The resulting polarization in adjacent cells is opposite to that within the stimulated cell, which may be indicative of lateral inhibition. Also such interactions promote distortion and suppression in the electrical and, consequently, the mechanical activity of OHCs. Lateral interactions may provide a basis for enhanced frequency selectivity in the basilar membrane of mammals.

The mechanical response of the OHC is mirrored by an electrical signature, a motility-related charge movement, similar to the gating charge movements that control ion-channel conductance^{12,13}. Both gating currents arise from a redistribution of charged voltage sensors across the membrane. The magnitude of the gating current reflects the rate of charge redistribution. In the OHC, the redistribution of motility-related charge is controlled by both voltage and membrane tension; consequently, either can evoke gating currents^{14–16}. Within the organ of Corti, OHCs are indirectly mechanically coupled to each other through contacts with supporting Deiters' cells. The apical regions of OHCs and Deiters' cells are joined by tight junctions and form the plate-like reticular lamina. Basally, the OHCs sit in the cups of Deiters' cells, and the strength of these attachments varies along the length of the basilar membrane¹⁷. This morphology makes it likely that the voltage-induced mechanical responses of one OHC will affect surrounding OHCs. Determining the nature of this interaction may provide insight into the process of fine frequency tuning by OHCs. We studied this interaction by simultaneously recording from adjacent OHCs under dual whole-cell voltage and current clamp.

In isolated pieces of Corti's organ, where cellular relations remain intact, voltage stimulation of an OHC induces mechanical responses and gating currents in that cell (Fig. 1). Transient outward currents are generated by the onset of depolarization, which causes the cell to contract (Fig. 1b). These currents correspond to the displacement of positive charge to the extracellular aspect of the lateral plasma membrane, which holds motility/voltage sensors^{18,19}. The charge–voltage ($Q-V$) function is well described by a two-state

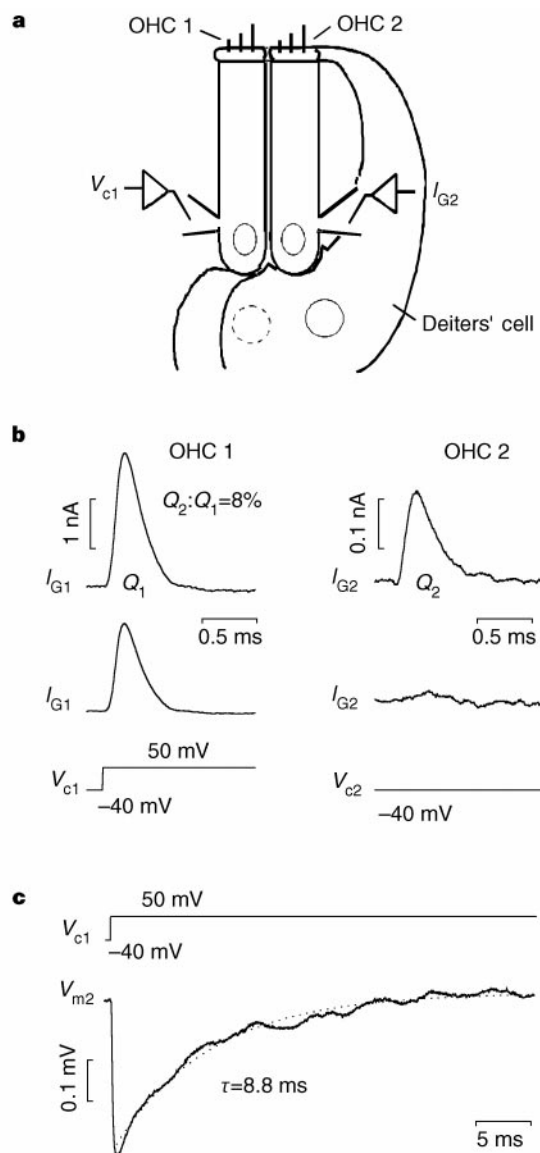


Figure 1 Adjacent OHCs are mechano-electrically coupled. **a**, Schematic illustrating two adjacent OHCs mechanically coupled through Deiters' cells and the recording configuration. **b**, Each cell in a pair was whole-cell voltage clamped to -40 mV. Depolarization of OHC 1 to 50 mV (V_{c1}) generates outward transient currents in both OHC 1 and OHC 2 (I_{G1} , I_{G2} , respectively; top two traces). The charge ratio (Q_2/Q_1) is 0.08 . Following application of negative pressure (~ -0.5 kPa; middle two traces) to the patch pipette of OHC 1, the response in OHC 2 was abolished. The response in the voltage-stimulated cell is decreased somewhat, as expected, owing to a negative shift in its Q - V function¹⁶. **c**, OHC 1 was held under voltage clamp and OHC 2 under current clamp. As a consequence of the impulsive gating current evoked in OHC 2, a negative voltage is developed across its membrane. The trace depicts the average induced potential ($n = 7$) in OHC 2. Dotted line: single exponential fit of 8.8 ms.

Boltzmann function^{12,13}:

$$Q(V) = \frac{Q_{\max}}{1 + b} \quad (1)$$

$$b = \exp\left(\frac{-ze(V_m - V_h)}{kT}\right)$$

where $Q_{\max}$ is the maximum nonlinear charge moved, V_h is the voltage at half-maximal gating-charge transfer, V_m is the membrane potential, z is the valence, e is the electron charge, k is Boltzmann's constant and T is the absolute temperature.

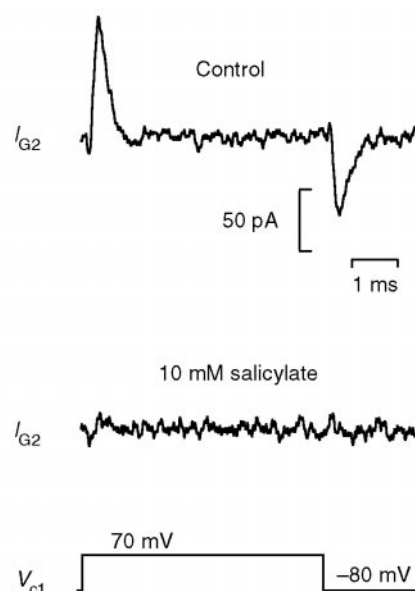


Figure 2 Both OHCs were voltage clamped to -80 mV, and OHC 1 was stepped to 70 mV (V_{c1}). The top trace shows the gating current (I_{G2}) evoked in OHC 2. Perfusion of the cells with 10 mM salicylate abolishes the gating current.

When the stimulated OHC (OHC 1) is depolarized, gating currents are seen in the adjacent OHC (OHC 2). The ratio of charge movement (Q_2/Q_1) in adjacent OHCs can be up to 0.1 . The induced gating current in OHC 2 is not generated by electrical interactions between the cells. Individually isolated cells that are positioned to touch each other do not show this charge coupling. Furthermore, collapse of OHC 1 by negative pipette pressure (-0.25 to -0.5 kPa), which abolishes the cell's normal mechanical activity¹³, eliminates gating currents in adjacent OHCs (Fig. 1b).

As in OHC 1, the magnitude of the gating currents in OHC 2 reflects the rate of charge redistribution; in this case, however, the rate mirrors that of the deformation of OHC 2. This is indicated by the significant correlation between the OHC 1 clamp time constant (which controls the rate of the OHC mechanical response under whole-cell voltage clamp⁶) and gating current magnitude in OHC 2 ($r^2 = 0.63$; $n = 21$). This is expected, because the OHC gating-current magnitude resulting from stretch is related to the velocity of stretch¹⁵. The charge redistribution in OHC 2 generates a voltage with a time course that depends on the cells' membrane time constant ($\tau_m = R_m \times C_m$). We looked at this induced voltage under current clamp. In seven cells, the average time constant of voltage decay induced by the impulsive gating current was 8.8 ms. The average membrane resistance of these cells was 272 ± 28 M Ω (mean $\pm$ s.e.), the membrane capacitance (at the holding potential) was 35.1 ± 2.02 pF and, consequently, $\tau_m = 9.5$ ms. The initial voltage magnitudes were up to 1.2 mV. The polarity of the induced voltage was negative, corresponding to the movement of positive charge to the outside of the cell membrane. The polarity of the voltage change in mechanically coupled cells is opposite to that of the electrically stimulated cell. Direct currents (d.c.) were not observed in adjacent OHCs, confirming the absence of gap-junction-mediated electrical coupling.

Additional evidence indicates that the transient currents and voltages in adjacent OHCs result from mechanical coupling between OHCs, and that these electrical responses are generated by the displacement of motility/voltage sensors. Figure 2 shows that salicylate, a known blocker of OHC gating currents and motility^{20,21}, abolishes the gating currents in adjacent OHCs. In our experiments, salicylate probably has two effects: it blocks both the mechanical response of the stimulated cell and the charge movement in the

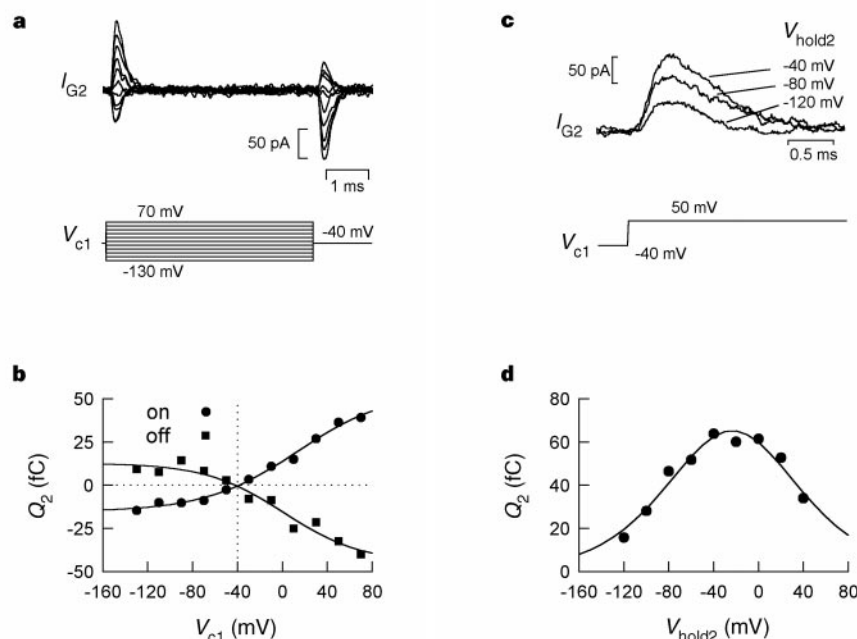


Figure 3 Gating currents are a function of both OHCs' holding potential. **a**, Both OHCs were voltage clamped to -40 mV, and OHC 1 was stepped for 5 ms from -130 to 70 mV (V_{c1}) in 20 mV steps. Gating currents (I_{G2}) in an adjacent OHC are shown in the top trace. The polarity of the currents depends on that of the stimulus voltage in OHC 1. **b**, the Q - V plot (on and off charge in OHC 2 versus voltage of OHC 1) shows a reversal of gating charge sign at the OHC 1 holding potential. The nonlinearity of the response partially reflects the nonlinear nature of the motility

function. **c**, The magnitude of gating current (I_{G2} , top traces) in OHC 2 induced by a fixed voltage step in OHC 1 depends on the membrane (holding) potential of OHC 2. **d**, The Q - V plot (on charge in OHC 2 versus holding voltage of OHC 2) shows that charge magnitude is a bell-shaped function of OHC 2 holding potential. The data were fit (solid line) to equation (2) with Q_{max} (prior determination from Q_2 - V_{c2} function) fixed at 2.99 pC. $\delta V_h = 2.5$ mV; $V_h = -23.5$ mV; $z = 0.65$.

adjacent cell. Further evidence derives from the polarity of the gating currents in adjacent cells (Fig. 3a, b). As expected for a process dependent on the stimulated OHC's mechanical response, the gating current polarity of the adjacent cell reverses at the holding potential of the stimulated cell; depolarization from this potential causes contraction of the cell, whereas hyperpolarization produces elongation. The deformation of adjacent cells will alter the tension on their lateral membranes. Reduced tension effectively shifts the voltage dependence of the Q - V function in the negative direction, and will evoke a positive gating current¹⁶. This agrees with the effects measured in adjacent OHCs. The magnitude of charge movement induced by this shift depends on the adjacent cell's membrane (holding) potential and is given by the difference between the two shifted Boltzmann functions¹⁵:

$$Q(V) = -Q_{max} \delta V_h \frac{ze}{kT(1+b)^2} \quad (2)$$

where δV_h is the magnitude of the V_h shift induced by a change in membrane tension, and b is as in equation (1).

Indeed, this behaviour is seen in our experiments (Fig. 3c, d). The magnitude of gating charge in the adjacent OHC depends on its holding potential, and the bell-shaped function of equation (2) satisfactorily describes the relationship. As the Q - V function mirrors the OHC's motility function¹⁶, the mechanical activity of coupled OHCs will be affected similarly.

Our data show that, as a result of OHC mechanical activity, lateral interactions exist within the organ of Corti that may significantly affect its responsiveness to sound. We have explored some of the consequences of these interactions. When two pure-tone frequencies are presented to a subject, auditory illusions of additional tonal frequencies and measurable physiological counterparts result^{22,23}. Additionally, one tone can be suppressed by another. This distortion arises from nonlinearities in the peripheral auditory system, and in the mammal it results from OHC activity^{24,25}. Even within single isolated hair cells, distortion evoked by two-frequency stimulation

can be generated by nonlinearities of the stereociliar transducer or by OHC motility²⁶⁻²⁸. We tested whether this type of distortion can arise from interactions between OHCs by simultaneously and independently stimulating each cell of coupled pairs with differing voltage frequencies (OHC 1, $f_1 = 813.8$ Hz; OHC 2, $f_2 = 976.5$ Hz) and observing nonlinear interactions. Under these stimulation conditions, both frequencies are present in the current responses of each cell (Fig. 4). The magnitude of the mechanically induced frequency component (for example, f_2 in OHC 1) is a bell-shaped function of holding potential, indicating, as above, that this component is generated by frequency-following shifts in the voltage dependence of the Q - V function of the cell. Furthermore, this mechanically generated frequency component suppresses the voltage-evoked frequency component by up to 10%. Finally, the interaction of the two frequency components gives rise to nonlinear intermodulation distortion in each cell (Fig. 4b). The sum and difference distortion components ($f_2 + f_1, f_2 - f_1$) can be observed as little as 20 dB below the mechanically induced primary.

Our data show that nonlinear mechanical interactions occur among OHCs within Corti's organ, and that these interactions generate distortion and suppression that are similar to those found in the intact organ. The generated distortion, seen only in coupled OHCs, may arise not only from the interaction of intrinsic cellular nonlinearities^{27,28}, but also from nonlinearity in the visco-elastic coupling between cells. The mechanical coupling strength, based on ratios of charge or current, is up to 10%. However, the degree of mechano-electrical coupling among OHCs may be greater *in vivo* than is demonstrated here, as physical coupling in our preparations was probably compromised by the isolation procedure.

It is believed that passive basilar-membrane tuning is enhanced by feedback from OHC mechanical activity³; however, based solely on mechanical activity evoked by passive tuning, sharpening would not be expected. The consequence of OHC coupling is twofold for adjacent cells; the voltage dependence of motility is altered, and a voltage of opposite phase is evoked. These mechanically generated

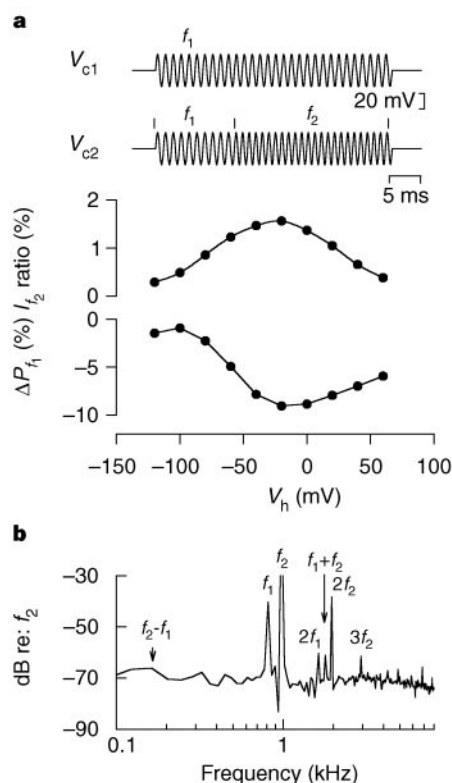


Figure 4 Both OHCs were voltage clamped to -40 mV and independently stimulated with pure sine-wave voltages. **a**, The upper two traces show the voltage stimuli delivered independently to each cell. During the initial part of the stimulus duration, each cell received a 20 mV peak sine wave at $f_1 = 813.8$ Hz; in the latter three-quarters of the stimulus duration, the OHC 2 frequency was switched to 976.5 Hz. Fast Fourier transform analysis was limited to the last half of stimulus duration to avoid transient responses. Comparisons were made before and after uncoupling by collapsing OHC 2. The upper plot shows the ratio of mechanically induced f_2 current (I_2) in OHC 1 due to f_2 voltage stimulation in OHC 2. The ratio of the power of the f_1 component in the response of OHC 1 before and after OHC 2 was collapsed was also obtained. The change in this ratio (ΔP_1) is shown in the lower plot. Negative percentage means that the f_1 response after collapse of OHC 2 was larger than that before; in other words, the mechanically induced f_2 component in OHC 1 suppressed the f_1 response in OHC 1. **b**, The spectrum of current response in OHC 2. The magnitude (in dB) is referenced to f_2 magnitude. Besides harmonic distortion products, the peak of sum frequency distortion ($f_1 + f_2$) is clearly visible.

lateral interactions may allow basilar membrane motion to be selectively enhanced at a particular location where passive vibration is maximal. In nonlinear systems, 10% feedback can have enormous consequences. The likely increase in OHC coupling with increasing frequency, supported by morphological evidence¹⁷, may explain the finding that tuning is sharper at higher characteristic frequencies. In this model, distortion in the cochlea arises from the very process that promotes greater frequency resolution. □

Methods

Pieces of the organ of Corti, containing between 3 and more than 50 OHCs with associated Deiters' cells, were freshly isolated from the guinea-pig cochlea, and adjacent OHCs were separately whole-cell voltage clamped at room temperature using an Axon 200A and 200B amplifier. Membrane voltages were corrected for the effects of residual series resistance, which ranged from 3 to 7 M Ω . We used ionic blocking solutions to remove voltage-dependent ionic conductances so that capacitive currents could be analysed in isolation²⁹. Gating currents were extracted using the P/4 technique³⁰. Pipette crosstalk artefacts at the onset of traces (first 50 μ s) were subtracted from the data, using the artefacts obtained in the absence of mechanical coupling. All data collection

and most analyses were performed with an in-house-developed, Windows-based whole-cell voltage-clamp program, jClamp (www.med.yale.edu/surgery/otolar/santos/jclamp.html), using a Digidata 1200 board (Axon).

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Signals from the reproductive system regulate the lifespan of *C. elegans*

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Understanding how the ageing process is regulated is a fascinating and fundamental problem in biology. Here we demonstrate that signals from the reproductive system influence the lifespan of the nematode *Caenorhabditis elegans*. If the cells that give rise to the germ line are killed with a laser microbeam, the lifespan of the

animal is extended. Our findings suggest that germline signals act by modulating the activity of an insulin/IGF-1 (insulin-like growth factor) pathway that is known to regulate the ageing of this organism. Mutants with reduced activity of the insulin/IGF-1-receptor homologue DAF-2 have been shown to live twice as long as normal¹⁻³, and their longevity requires the activity of DAF-16, a member of the forkhead/winged-helix family of transcriptional regulators^{1,2,4,5}. We find that, in order for germline ablation to extend lifespan, DAF-16 is required, as well as a putative nuclear

hormone receptor, DAF-12 (refs 6, 7). In addition, our findings suggest that signals from the somatic gonad also influence ageing, and that this effect requires DAF-2 activity. Together, our findings imply that the *C. elegans* insulin/IGF-1 system integrates multiple signals to define the animal's rate of ageing. This study demonstrates an inherent relationship between the reproductive state of this animal and its lifespan, and may have implications for the co-evolution of reproductive capability and longevity.

When *C. elegans* hatches, its gonad consists of four precursor

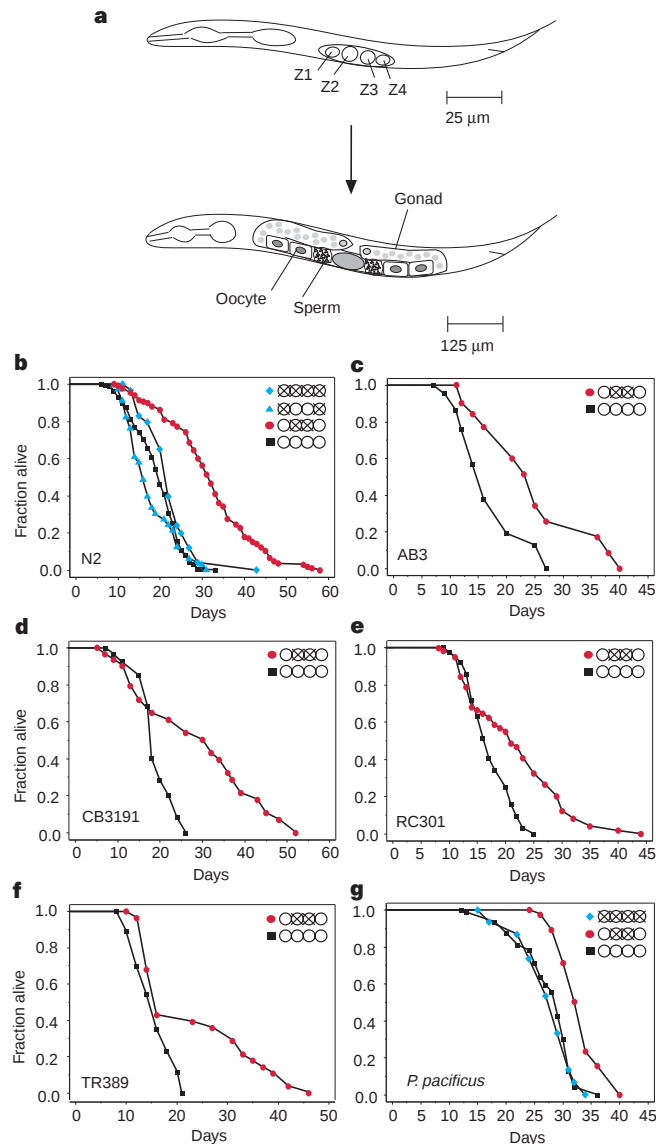


Figure 1 Response of wild-type nematodes to ablation of germline and somatic-gonad precursors. **a**, The *C. elegans* gonad. At hatching (top panel), the gonad consists of four cells. Z1 and Z4 give rise to the somatic gonad, and Z2 and Z3 give rise to the germ line of the adult (lower panel). Wild-type hermaphrodites have approximately 300 progeny; however, progeny production does not affect lifespan, since *fem* mutants (which lack sperm) have normal lifespans (also see text)¹⁷. The somatic gonad is needed for germline development¹⁴, and in all of our experiments, ablating only the somatic gonad had the same effect as ablating both the somatic gonad and germ line. **b-g**, Survival curves. The curves shown represent the sum of all animals examined in one or more experiments (each experiment consisting of sets of ablated animals and control animals that were born at the same time). *n*, total number of animals observed (the number of independent experiments performed is given in parentheses); *m*, mean lifespan; -, ablated. *P* values were calculated between the experimental and control animals examined in a single experiment. **b**, Wild type (strain N2). Intact control, *n* = 438(10), *m* = 19.4 ± 0.29; Z2/3(-), *n* = 146(7), *m* = 31.8 ± 0.97, *P* ≤ 0.0001

for each of 6 experiments (in the 7th, *P* = 0.0023); Z1/2/3/4(-), *n* = 34(1), *m* = 22.5 ± 1.16, *P* = 0.33 (similar results have been reported previously¹); Z1/4(-), *n* = 34(2), *m* = 17.8 ± 1.00, *P* = 0.87 (experiment 1) and 0.35 (experiment 2). A second laboratory strain of N2, MRC N2, responded similarly to Z2/3 and Z1/4 ablation (data not shown). **c-f**, Survival of wild isolates of *C. elegans* following Z2/3 ablation. **c**, AB3 is an Australian strain. Intact control, *n* = 35(1), *m* = 17.1 ± 1.33; Z2/3(-), *n* = 32(1), *m* = 25.1 ± 2.44, *P* = 0.0056. **d**, CB3191 is an isolate from California. Intact control, *n* = 33(1), *m* = 19.0 ± 0.80; Z2/3(-), *n* = 31(1), *m* = 29.0 ± 2.60, *P* = 0.0004. **e**, RC301 is a German strain. Intact control, *n* = 72(2), *m* = 17.2 ± 0.63; Z2/3(-), *n* = 69(2), *m* = 21.8 ± 1.15, *P* = 0.16 and 0.0034. **f**, TR389 is from Wisconsin. Intact control, *n* = 30(1), *m* = 15.6 ± 0.71; Z2/3(-), *n* = 31(1), *m* = 23.4 ± 2.09, *P* = 0.0012. AB3 and RC301 exhibit clumping behaviour, the other strains do not⁹. **g**, *P. pacificus* (family Diplogastridae). *n* = 83(2), *m* = 27.5 ± 0.82; Z2/3(-), *n* = 39(1), *m* = 33.3 ± 0.69, *P* < 0.0001; Z1/2/3/4(-), *n* = 29(1), *m* = 27.6 ± 1.14, *P* = 0.64.

cells, called Z1, Z2, Z3 and Z4 (ref. 8). Z1 and Z4 give rise to the somatic gonad, and Z2 and Z3 give rise to the germ line (Fig. 1a). Previous studies have shown that if the entire gonad (Z1/2/3/4) is ablated with a laser microbeam at hatching, lifespan is unaffected; this suggests that the production of progeny *per se* does not influence the ageing process¹. However, we found that if we ablated the germline precursor cells at hatching but left the somatic-gonad precursors intact, the animals remained active and healthy longer than normal and lived approximately 60% longer (Fig. 1b). Thus, in normal animals, a signal that depends on the germ line accelerates ageing.

To determine whether this phenomenon was confined to laboratory strains of *C. elegans*, we examined various wild *C. elegans* strains from around the world⁹. We found that all these strains lived longer after ablation of the germline precursors (Fig. 1c–f). We also ablated the germline precursors in *Pristionchus pacificus*, a nematode species thought to have diverged from *C. elegans* more than 100 million years ago¹⁰, and found that its lifespan also increased (Fig. 1g). This phenomenon may therefore be conserved among different nematode families.

We next investigated which genes might be involved in this signalling system. *daf-16* activity is known to be required for the longevity of *daf-2* mutants. We found that *daf-16* is also required for the longevity of germline-ablated animals, because ablating the germline precursors in *daf-16* null mutants had no effect on lifespan (Fig. 2a). *daf-16* mutants do not have long lifespans, as would be expected if they were defective in the production or activity of the germline signal. Thus, we favour the interpretation that in normal animals, signals from the germ line inhibit DAF-16 activity. When the germline precursors are ablated, DAF-16 activity rises and lifespan is extended.

As both *daf-2* mutations and germline ablation increase lifespan, we investigated whether their effects might be additive or synergistic by ablating the germline precursor cells in four *daf-2* mutants, *e1370*, *m596*, *e1368* and *m41* (ref. 11). In each case, lifespan was extended dramatically (Fig. 3a–d); on average, the germline-ablated animals lived almost twice as long as intact *daf-2* controls, that is, almost four times as long as wild-type animals. Some of these animals lived as long as four months, remaining active much longer than intact *daf-2* animals.

Because *daf-2* mutations and germline ablation had synergistic effects on lifespan, germline signals and DAF-2 activity may act in parallel to shorten lifespan by downregulating DAF-16 activity. Thus, when either DAF-2 activity or germline signalling is

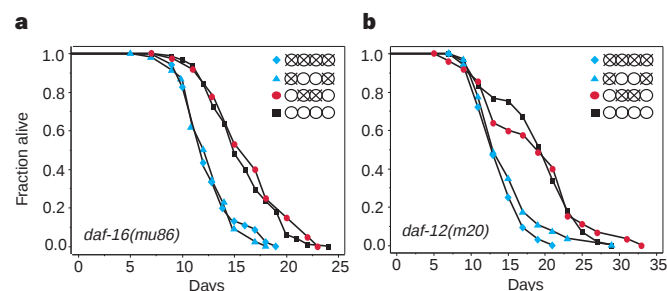


Figure 2 Responses of *daf-16* and *daf-12* mutants to ablation of germline and somatic-gonad precursors. **a**, Survival curves of the *daf-16* null mutant *mu86* (ref. 4). Intact controls, $n = 148(4)$, $m = 15.8 \pm 0.30$; $Z2/3(-)$, $n = 42(1)$, $m = 16.6 \pm 0.68$, $P = 0.78$; $Z1/2/3/4(-)$, $n = 53(2)$, $m = 12.9 \pm 0.34$, $P = 0.0018$ and 0.0095 in each of two experiments; $Z1/4(-)$, $n = 45(2)$, $m = 12.6 \pm 0.36$, $P = 0.014$ and <0.0001 . Ablating Z1 and Z4 also shortened the lifespans of two other *daf-16* alleles, *m26* and *m27*; ablating Z2 and Z3 in these animals did not significantly affect lifespan (data not shown). **b**, A *daf-12* putative null allele, *m20* (ref. 11). Intact control, $n = 105(3)$, $m = 19.2 \pm 0.61$; $Z2/3(-)$, $n = 51(3)$, $m = 18.7 \pm 0.97$, $P = 0.70$, 0.89 and 0.46 ; $Z1/2/3/4(-)$, $n = 32(1)$, $m = 14.0 \pm 0.52$, $P < 0.0001$; $Z1/4(-)$, $n = 34(1)$, $m = 15.0 \pm 0.77$, $P = 0.0006$.

decreased, DAF-16 activity rises, and when both are decreased, DAF-16 activity rises even further. As these *daf-2* mutations are not null alleles¹¹, it is also possible that ablating the germline precursors of *daf-2* mutants increases lifespan by downregulating residual DAF-2 activity. We consider this interpretation less likely, however, because double mutants carrying mutations in *daf-2* and another gene thought to function in the *daf-2* pathway, the phosphatidylinositol-3-OH kinase *age-1* (ref. 12), have lifespans similar to those of *daf-2* single mutants^{2,13}.

In the wild type, the somatic gonad must be present for germline ablation to extend lifespan¹ (Fig. 1b). This is consistent with two models. First, germline cells could produce a signal that shortens lifespan while the somatic gonad produces a different signal that

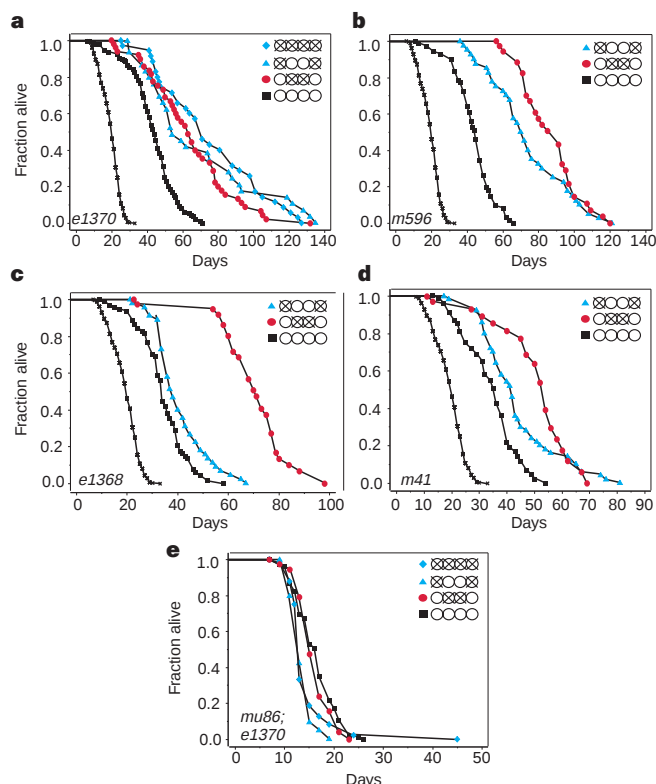


Figure 3 Responses of *daf-2* mutants to ablation of the germline and somatic-gonad precursors. **a–d**, Survival curves of intact N2 animals (left) are shown for comparison. **a**, Survival curve of *daf-2(e1370)*. Intact control, $n = 245(5)$, $m = 43.2 \pm 0.93$; $Z2/3(-)$, $n = 59(2)$, $m = 64.5 \pm 3.47$, $P = 0.092$ (in an experiment in which $n = 13$) and <0.0001 . $Z1/2/3/4(-)$, $n = 40(1)$, $m = 75.7 \pm 4.68$, $P = 0.079$ vs the germline-ablated group, $P < 0.0001$ vs the control group. $Z1/4(-)$, $n = 31(2)$, $m = 69.5 \pm 6.02$, $P = 0.33$ vs germline-ablated animals, $P = 0.019$ and <0.0001 vs control. **b**, *daf-2(m596)*. Intact control, $n = 89(2)$, $m = 43.9 \pm 1.34$; $Z2/3(-)$, $n = 40(1)$, $m = 86.9 \pm 2.82$, $P < 0.0001$. $Z1/4(-)$, $n = 43(1)$, $m = 73.9 \pm 3.43$, $P = 0.077$ vs germline-ablated, $P < 0.0001$ vs control. **c**, *daf-2(e1368)*. Intact control, $n = 110(3)$, $m = 34.3 \pm 1.01$; $Z2/3(-)$, $n = 45(1)$, $m = 71.0 \pm 2.27$, $P < 0.0001$. $Z1/4(-)$, $n = 45(2)$, $m = 41.1 \pm 1.47$, $P < 0.0001$ vs germline-ablated (for both experiments); $P < 0.0001$ (experiment 1, $n = 17$, $m = 49.1 \pm 2.25$) and $P = 0.43$ (experiment 2, $n = 28$, $m = 36.2 \pm 1.22$) vs control. **d**, *daf-2(m41)*. Intact control, $n = 90(2)$, $m = 34.3 \pm 1.20$; $Z2/3(-)$, $n = 36(1)$, $m = 51.1 \pm 2.48$, $P < 0.0001$. $Z1/4(-)$, $n = 51(2)$, $m = 43.9 \pm 1.91$, $P = 0.06$ vs germline-ablated, $P = 0.01$ and 0.02 vs control. The DNA sequences of the *m596* and *m41* mutations are not known. **e**, Survival curves of *daf-16(mu86); daf-2(e1370)* double mutants. Intact animals, $n = 155(4)$, $m = 16.4 \pm 0.38$; $Z2/3(-)$, $n = 40(1)$, $m = 16.2 \pm 0.60$, $P = 0.03$ (this may not be significant since $P = 0.52$ if 91 additional control animals cultured but not born in parallel are included in the data set); $Z1/2/3/4(-)$, $n = 50(2)$, $m = 14.6 \pm 0.77$, $P = 0.011$ and 0.24 ; $Z1/4(-)$, $n = 46(1)$, $m = 13.7 \pm 0.31$, $P = 0.0081$.

lengthens lifespan. Second, germline ablation could cause the somatic gonad, which is normally silent, to emit a signal that extends lifespan. In principle, these two models could be distinguished by ablating the somatic-gonad precursors but not the germline precursors. Model 1 predicts that the animals would have short lifespans and model 2 that they would have normal lifespans. Unfortunately, this experiment is not possible because the germ line depends on the somatic gonad for its development¹⁴. However, we found that ablating the whole gonads of *daf-16* mutants shortened their lifespans relative to intact *daf-16* controls. This finding leads us to favour model 1, in which the germ cells and somatic gonad have opposite effects on lifespan. If *daf-16* mutants cannot respond to germline ablation but can respond to somatic-gonad ablation, killing the whole gonad would shorten lifespan (Fig. 2a).

We next investigated what gene activities might cause somatic-gonad ablation to shorten the lifespans of germline-ablated animals. One gene known to shorten lifespan is wild-type *daf-2*. Therefore, we asked whether the lifespan extension produced in *daf-2* mutants by ablating the germline precursors could be suppressed by ablating the somatic-gonad precursors. We found that, unlike in wild type, in two *daf-2* mutants, *e1370* and *m596*, it could not, since ablating either the germline precursors alone or both the germline and somatic-gonad precursors extended their lifespans (Fig. 3a, b). This suggests that the response to somatic-gonad ablation requires *daf-2*(+) activity. A simple model for DAF-2's role is that, in normal animals, signals from the somatic gonad promote longevity by inhibiting DAF-2 activity. When the somatic gonad is ablated, DAF-2 activity rises and lifespan is shortened.

Surprisingly, in a different *daf-2* mutant, *e1368*, ablation of the somatic gonad almost completely suppressed the effect of ablating the germ line, as occurs in wild-type animals (Fig. 3c). Thus, this mutation seemed to uncouple two lifespan-shortening activities of DAF-2. Like other *daf-2* mutations, the *e1368* mutation increases

the lifespans of animals with intact gonads; therefore, it interferes with a gonad-independent activity of DAF-2 that reduces lifespan. However, unlike *daf-2*(*e1370*), the *e1368* mutation did not abolish the response to somatic-gonad ablation. We saw a similar but less striking effect with the *m41 daf-2* mutant, which had a response intermediate between those of *e1370* and *m596* and that of *e1368* (Fig. 3d).

One possible reason that *e1368* behaves differently from other *daf-2* alleles is suggested by the molecular nature of these mutations. The *daf-2*(*e1370*) mutation, which blocks both *daf-2* activities, affects the putative tyrosine kinase domain of DAF-2 (ref. 3), whereas *e1368*, which affects only the gonad-independent activity, affects the putative ligand-binding domain³. Because intact *e1368* animals are long-lived, this lesion seems likely to prevent the DAF-2 protein from responding to an insulin-like ligand that is produced independently of the gonad. However, the fact that *e1368* can still respond to somatic-gonad ablation raises the possibility that this mutant DAF-2 protein retains the ability to respond to a second insulin-like ligand whose production depends on the somatic gonad. At present, no DAF-2 ligands have been identified, but the *C. elegans* genome contains a number of insulin/IGF-1-like sequences¹⁵.

Another gene that can affect *C. elegans* lifespan is *daf-12*, which encodes a putative nuclear hormone receptor^{6,7}. Mutations in *daf-12* slightly shorten the lifespans of otherwise wild-type animals^{2,11} and also affect the lifespans of *daf-2* mutants (see below). We ablated the germline precursors in animals carrying *daf-12*(*m20*), a putative null allele¹¹, and found that this did not affect lifespan (Fig. 2b). Thus, *daf-12* activity is required for germline ablation to extend lifespan. We also ablated the whole gonads of *daf-12* mutants and found that their lifespan was shortened (Fig. 2b). This finding is consistent with the model that DAF-12, like DAF-16, functions in the germline but not the somatic-gonad signalling pathway.

The *daf-12*(*m20*) mutation is known to lengthen the lifespans of *daf-2*(*e1370*) mutants but not those of *daf-2*(*m41*) mutants^{2,11}. Similarly, loss of the whole gonad lengthens the lifespans of *daf-2*(*e1370*) mutants, but has a milder effect on those of *daf-2*(*m41*) mutants (Fig. 3a, d). The similar effects of whole-gonad ablation and *daf-12* mutations suggest that in *daf-2* mutants, *daf-12* activity could be required for signalling from both the somatic gonad and the germ line. However, it is difficult to reconcile this hypothesis with the finding that *daf-12* is not required for somatic-gonad signalling in an otherwise wild-type background. Perhaps DAF-12 activity is influenced by DAF-2.

Together, our findings can be explained by several models, but because of its relative simplicity we favour the model shown in Fig. 4a. In normal animals an insulin-like, gonad-independent ligand activates DAF-2, which in turn downregulates DAF-16 and shortens lifespan. Signals from the germ line also shorten lifespan by downregulating a pathway involving both DAF-16 and DAF-12. If germline signalling is inhibited, the activity of this DAF-16/DAF-12-dependent pathway rises and lifespan is increased. In addition, the somatic gonad produces a signal that lengthens lifespan by inhibiting DAF-2 activity. This somatic-gonad signal may be, or may control, a second insulin-like DAF-2 ligand. If somatic-gonad signalling is inhibited, changes in the level of this ligand lead to increased DAF-2 activity and shortened lifespan. Thus, in this model the reciprocal activities of germline and somatic-gonad signals arise because one signal acts on DAF-16, which lengthens lifespan, and the other acts on DAF-2, which shortens lifespan.

This model predicts that ablating germline or somatic-gonad precursors in *daf-16*; *daf-2* double mutants would have no effect. We found that ablating the germline precursors had no apparent effect, but that ablating the whole gonad shortened lifespan in a small but statistically significant way (Fig. 3e). This suggests that other factors may also contribute to this pathway.

We also investigated whether the lifespan of *C. elegans* was sensitive to quantitative differences in the level of somatic-gonad

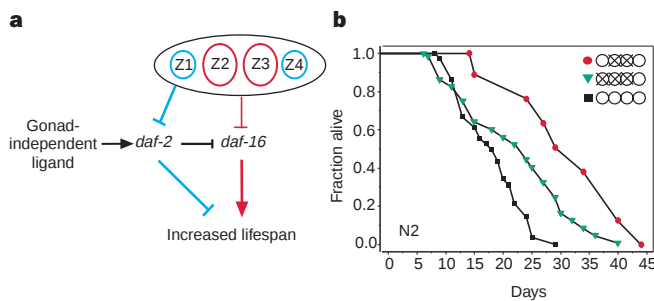


Figure 4 A model for the effects of reproductive signals on lifespan. **a**, In this model, two types of gonad-dependent signals influence lifespan. A signal from the germ cells decreases lifespan by downregulating the activity of *daf-16* (red lines) and *daf-12* (not shown). A counterbalancing signal from the somatic gonad increases lifespan by downregulating *daf-2* activity (blue lines). The somatic-gonad signal may be, or may control, a second insulin-like ligand for *daf-2*. In addition to signals from the reproductive system, a gonad-independent signal shortens lifespan by activating *daf-2*, which in turn downregulates *daf-16* activity. The site of integration of the three signals is not known. In principle, all these signals could act on the same cells, or different signals could act on different cells. Finally, we note that although both *daf-16* and *daf-12* function in the germline signalling pathway, only *daf-16* is absolutely required for the gonad-independent signalling pathway. In addition, *daf-12* may possibly have a role in somatic-gonad signalling that is influenced by *daf-2* (not shown, see text). **b**, Ablation of only one of the two somatic-gonad precursor cells does not fully suppress the effect of ablating the germline precursors ($P = 0.014$ between $Z1/2/3(-)$ [$n = 38$, $m = 22.0 \pm 1.75$] and $Z2/3(-)$ [$n = 10$, $m = 31.0 \pm 3.30$]; $P = 0.013$ between $Z1/2/3(-)$ and intact control [$n = 40$, $m = 18.0 \pm 0.90$]). This suggests that the system is sensitive to the quantity of somatic-gonad signalling. Because the germline lineage is variable^{8,14}, it is not clear what effect ablating only one of the two germline precursors would have on germline development and final germ-cell number.

signalling. We found that ablating one somatic-gonad precursor did not suppress the lifespan extension of germline-ablated animals to the same extent as ablating both precursors (Fig. 4b). Thus it seems likely that the animal can adjust its rate of ageing to the level of gonad signalling.

In addition to its role in ageing, the DAF-2/DAF-16 pathway controls the development of *C. elegans*⁷. When food is limiting, newly hatched animals do not progress to adulthood, but instead arrest development as reproductively immature dauer larvae until food is restored⁷. *daf-2* activity is required for animals to progress to adulthood (strong *daf-2* mutants form dauers constitutively), and *daf-16* activity is required for dauer formation (*daf-16* mutants cannot form dauers)⁷. Because of its role in dauer formation and because it encodes an insulin-receptor homologue, *daf-2* has been hypothesized to regulate the animal's development and rate of ageing in response to food signals, although this hypothesis has not been tested directly. In the present study, we have found that *daf-2* is also involved in a signalling pathway with a very different origin, the reproductive system. Thus, we speculate that the DAF-2/DAF-16 system may act globally to regulate the development and ageing of the animal in response to environmental signals such as food as well as internal signals reflecting the state of its reproductive system.

The signalling system described here has the interesting effect of placing the ageing process partially under the control of the germ line. This coupling could potentially have a beneficial effect on progeny production if the animal can monitor the state of its reproductive system and adjust its rate of ageing accordingly. In addition, coupling germline signals to lifespan could allow the processes of reproduction and ageing to co-evolve. For example, a mutation that slowed the growth of the germ line might simultaneously slow the animal's rate of ageing, enabling it to remain healthy and youthful long enough to produce progeny. □

Methods

Laser ablations. Ablations were performed on animals born within an hour of each other as described previously¹⁶. Survival of cells surrounding the gonad was monitored during the surgery, and successful ablation was confirmed by examining the germ cells and vulva under a dissecting microscope at the adult stage. The controls were grown in parallel with experimental animals and included some that were anaesthetized (1.0–1.5 M NaN₃) in parallel with ablated animals and some that were not. Anaesthesia had no effect on lifespan.

Lifespan analysis. Lifespans were determined at 20°C as described previously¹. The animals were cultured on standard agar plates (about five animals per plate). Day of birth was used as the first time point. All strains reached adulthood by 3 days after hatching, except for *daf-2(m41)* animals, which reached adulthood 2 days later (see ref. 11). Animals were considered dead when they ceased moving and responding to prodding. If they crawled off the plate, 'exploded' or died as 'bags of worms', they were censored at the last day of life. This step incorporated those worms into the data set until their censor date. The following are censored proportions in all groups examined, where a minus sign signifies ablated: 117/438 (N2 control), 7/34 [N2 Z1/2/3/4(-)], 11/38 [N2 Z1/2/3(-)], 1/34 [N2 Z1/4(-)], 38/146 [N2 Z2/3(-)], 18/35 (AB3 control), 19/32 [AB3 Z2/3(-)], 8/33 (CB3191 control), 3/31 [CB3191 Z2/3(-)], 38/72 (RC301 control), 17/69 [RC301 Z2/3(-)], 4/30 (TR389 control), 3/31 [TR389 Z2/3(-)], 56/83 (*P. pacificus* control), 14/29 [*P. pacificus* Z1/2/3/4(-)], 13/39 [*P. pacificus* Z2/3(-)], 38/148 [*daf-16(mu86)* control], 3/53 [*daf-16(mu86)* Z1/2/3/4(-)], 1/45 [*daf-16(mu86)* Z1/4(-)], 18/42 [*daf-16(mu86)* Z2/3(-)], 67/245 [*daf-2(e1370)* control], 5/40 [*daf-2(e1370)* Z1/2/3/4(-)], 2/31 [*daf-2(e1370)* Z1/4(-)], 13/59 [*daf-2(e1370)* Z2/3(-)], 27/89 [*daf-2(m596)* control], 3/43 [*daf-2(m596)* Z1/4(-)], 10/40 [*daf-2(m596)* Z2/3(-)], 34/110 [*daf-2(e1368)* control], 0/45 [*daf-2(e1368)* Z1/4(-)], 13/45 [*daf-2(e1368)* Z2/3(-)], 36/90 [*daf-2(m41)* control], 1/51 [*daf-2(m41)* Z1/4(-)], 15/36 [*daf-2(m41)* Z2/3(-)], 40/105 [*daf-12(m20)* control], 0/32 [*daf-12(m20)* Z1/2/3/4(-)], 4/34 [*daf-12(m20)* Z1/4(-)], 6/51 [*daf-12(m20)* Z2/3(-)], 49/155 [*daf-16(mu86)*; *daf-2(e1370)* control], 2/50 [*daf-16(mu86)*; *daf-2(e1370)* Z1/2/3/4(-)], 3/46 [*daf-16(mu86)*; *daf-2(e1370)* Z1/4(-)], 14/40 [*daf-16(mu86)*; *daf-2(e1370)* Z2/3(-)]. Survival curves, mean lifespans and *P* values were deter-

mined non-parametrically; log-rank tests were used to assess the similarity between two groups. Statview 4.5 software (Abacus) was used for all analyses.

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The homeobox gene *Phox2b* is essential for the development of autonomic neural crest derivatives

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The sympathetic, parasympathetic and enteric ganglia are the main components of the peripheral autonomic nervous system¹, and are all derived from the neural crest². The factors needed for these structures to develop include the transcription factor Mash1 (refs 3–5), the glial-derived neurotrophic factor GDNF (refs 6–8) and its receptor subunits^{9–12}, and the neuregulin signalling system¹³, each of which is essential for the differentiation and survival of subsets of autonomic neurons. Here we show that all autonomic ganglia fail to form properly and degenerate in mice lacking the homeodomain transcription factor *Phox2b*, as do the three cranial sensory ganglia that are part of the autonomic reflex circuits. In the anlagen of the enteric nervous system and the sympathetic ganglia, *Phox2b* is needed for the expression of the GDNF-receptor subunit Ret and for maintaining Mash1 expression. Mutant ganglionic anlagen also fail to switch on the genes that encode two enzymes needed for the biosynthesis of the neurotransmitter noradrenaline, dopamine-β-hydroxylase and

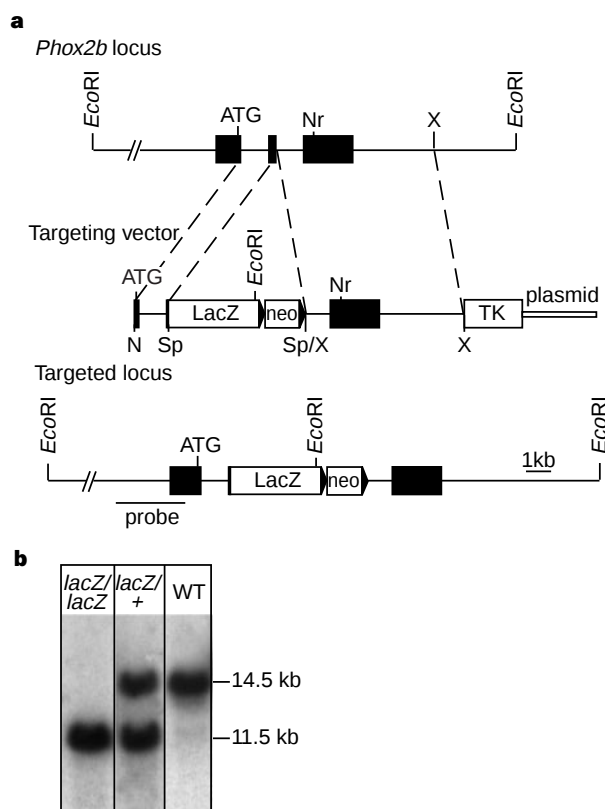


Figure 1 Targeted disruption of the *Phox2b* gene. **a**, Targeting scheme. Top: genomic organization of the *Phox2b* locus with exons in black. Middle: structure of the targeting vector. Bottom: predicted structure of the mutant allele. The probe used for Southern blot analysis is indicated. Restriction enzymes: N, *Not*I; Nr, *Nru*I; Sp, *Spe*I; X, *Xba*I. **b**, Southern blot analysis of DNA isolated from mutant homozygous, heterozygous and wild-type embryos, digested with *Eco*RI. The probe indicated in **a** detects a 14.5-kb and an 11.5-kb fragment, coming from the wild-type and mutant alleles, respectively. ATG, initiating codon.

tyrosine hydroxylase, demonstrating that *Phox2b* regulates the noradrenergic phenotype in vertebrates.

Phox2a and *Phox2b* are closely related neuronal type-specific paired-homeodomain transcription factors that are expressed throughout the developing sympathetic, parasympathetic and enteric ganglia^{14,15}. *Phox2b* expression starts as soon as the sympathoblasts aggregate on each side of the dorsal aorta and as the enteroblasts invade the foregut mesenchyme, and *Phox2a* expression starts soon after this. However, apart from the parasympathetic ganglia of the head¹⁶, these structures are not affected by the inactivation of the *Phox2a* gene. To investigate the role of *Phox2b* in the development of autonomic ganglia, we inactivated the gene by targeted insertion of a *LacZ* transgene in the second exon (Fig. 1). This insertion disrupts the homeobox of *Phox2b* and is expected to lead to a null phenotype. Heterozygous animals showed no obvious phenotype, but no homozygous mutants were born. In contrast, a mendelian proportion of *Phox2b*^{LacZ/LacZ} embryos was recovered at embryonic day 10.5 (E10.5) from intercrosses of heterozygotes.

The expression pattern of β -galactosidase (β -Gal) activity in heterozygotes was indistinguishable from that of the *Phox2b* locus (not shown) and is a reliable marker for mutant cells that should express *Phox2b*. In E13.5 homozygous *Phox2b*^{LacZ/LacZ} embryos, there were no cells expressing β -Gal at sites where the superior cervical ganglion (SCG) and the sympathetic chains are found in heterozygous embryos (Fig. 2a, c; and data not shown). Similarly, parasympathetic ganglia were undetectable (Fig. 2e, f; and data not shown) and no cells expressing β -Gal were found in the gut of homozygotes (Fig. 2i, j). The anlage of the mutant adrenal medulla

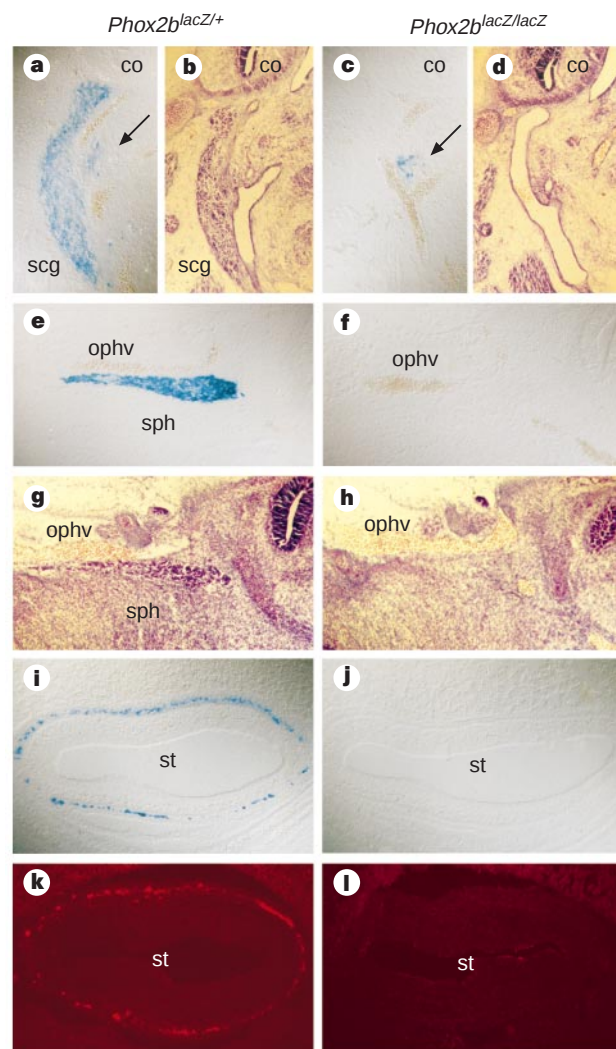


Figure 2 Absence of the sympathetic, parasympathetic and enteric components of the autonomic nervous system in E13.5 *Phox2b*^{LacZ/LacZ} embryos. **a-d**, X-gal stain (**a**, **c**) and Nissl stain (**b**, **d**) on parasagittal sections at the level of the SCG of heterozygous (left) and homozygous (right) *Phox2b*^{LacZ} embryos. Arrows in **a** and **c** point to cells belonging to the petrosal complex. **e-h**, X-gal stain (**e**, **f**) and Nissl stain (**g**, **h**) on parasagittal sections at the level of the sphenopalatine ganglion of heterozygous (left) and homozygous (right) *Phox2b*^{LacZ} embryos. **i-l**, X-gal stain (**i**, **j**) and immunofluorescence with anti-neurofilament-heavy-chain antibodies (**k**, **l**) on sagittal sections through the stomach of heterozygous (left) and homozygous (right) *Phox2b*^{LacZ} embryos. co: Cochlea; ophv: ophtalmic vein; scg: superior cervical ganglion; sph: sphenopalatine ganglion; st: stomach.

seemed morphologically intact at this stage and contained cells expressing β -Gal (not shown), but did not express the appropriate markers (see below). The lack of β -Gal expression at the sites of aggregation of autonomic ganglia could result from the absence of the cells or from premature loss of expression of the *LacZ* transgene. Histological analysis confirmed the absence of the sympathetic and parasympathetic ganglia (Fig. 2b, d, g, h). In the enteric nervous system, where the scattering of neuronal precursors precludes their detection by histological analysis at this stage, we could not detect expression of neurofilament heavy chain (Fig. 2k, l). These data strongly indicate that neurons or neuronal precursors are absent at all sites of formation of autonomic ganglia in E13.5 *Phox2b*^{LacZ/LacZ} embryos. In contrast, the sensory neuronal derivatives of the neural crest, which do not express *Phox2* genes, were not affected by the mutation (not shown).

To analyse the developmental abnormalities of these structures,

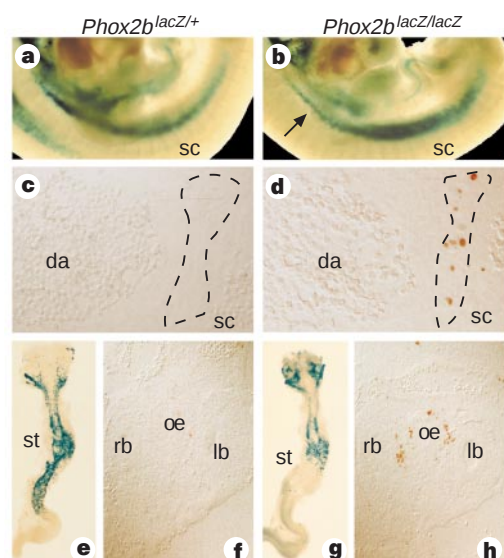


Figure 3 Early formation and degeneration of the sympathetic chain and the foregut enteric nervous system in *Phox2b^{LacZ/LacZ}* mice. **a, b**, Whole-mount X-gal stain of heterozygous (**a**) and homozygous (**b**) *Phox2b^{LacZ}* E10.5 embryos showing the early aggregation of the sympathetic chain in the absence of *Phox2b*. The arrow in **b** points to a reduced number of cells in the rostral part of the chain compared to the control. **c, d**, TUNEL analysis of transverse sections through the sympathetic chain in heterozygous (**c**) and homozygous (**d**) mutant embryos at E11.5. Apoptotic cells are detected only in the *Phox2b^{LacZ/LacZ}* mice. Dashed lines show the limits of the ganglion anlage, as revealed by *LacZ* expression on adjacent sections (not shown). **e, g**, Whole-mount X-gal stain on dissected guts from E10.5 heterozygous (**e**) and homozygous (**g**) *Phox2b^{LacZ}* embryos. In the control, β -Gal⁺ cells are detected in the foregut and hindgut, whereas in homozygous mutants only cells invading the foregut are visible. **f, h**, TUNEL analysis of transverse sections through the oesophagus of wild-type (**f**) and homozygous (**h**) *Phox2b^{LacZ}* embryos at E10.5. da: Dorsal aorta; lb, rb: left and right bronchus; oe: oesophagus; sc: sympathetic chain; st: stomach.

we examined embryos at around E10.5, shortly after the onset of formation of the sympathetic chain and the enteric nervous system. At the sites of formation of the primary sympathetic chain alongside the dorsal aorta, we found a pattern of β -Gal⁺ cells similar to that in heterozygotes, although there were fewer cells and they were more loosely organized (Figs 3a, b and 4a, b). We confirmed the presence of neural-crest cells alongside the dorsal aorta in mutant animals using the general neural-crest marker *Sox10*^{17–19} (Fig. 4c, d), which is normally co-expressed with *Phox2b* in all sympathetic ganglionic cells at E10.5 (data not shown). Therefore, in *Phox2b^{LacZ/LacZ}* mice, neural-crest-derived sympathetic progenitors aggregate at the sites of sympathetic ganglion formation, but subsequently fail to proliferate and/or degenerate. Consistent with this, numerous apoptotic figures were detected by TUNEL²⁰ analysis at E11.5 in the sympathetic chain of mutant, but not heterozygous, embryos (Fig. 3c, d).

Whole-mount preparations of the gut of E10.5 *Phox2b^{LacZ/+}* embryos showed that it was invaded by β -Gal⁺ cells down to the post-umbilical level, in agreement with the chronological appearance of enteric neural precursors expressing *Phox2b* (ref. 21) (Fig. 3e). In homozygous mutants, however, β -Gal⁺ cells were only present in the foregut, up to a sharp demarcation at the proximal stomach (Fig. 3g). The pattern was identical with *Sox10* (Fig. 5a, b, g, h; and data not shown). Therefore, in *Phox2b^{LacZ/LacZ}* mice, enteric-neuron precursors arrive at the foregut but fail to migrate further. This phenotype is very similar, as this stage, to that of *Ret*^{−/−} knockout mice¹⁰. In E13.5 embryos, even the foregut is devoid of neurons. We therefore assessed the fate of foregut enteric neuroblasts by TUNEL analysis. At E10.5, there were virtually no

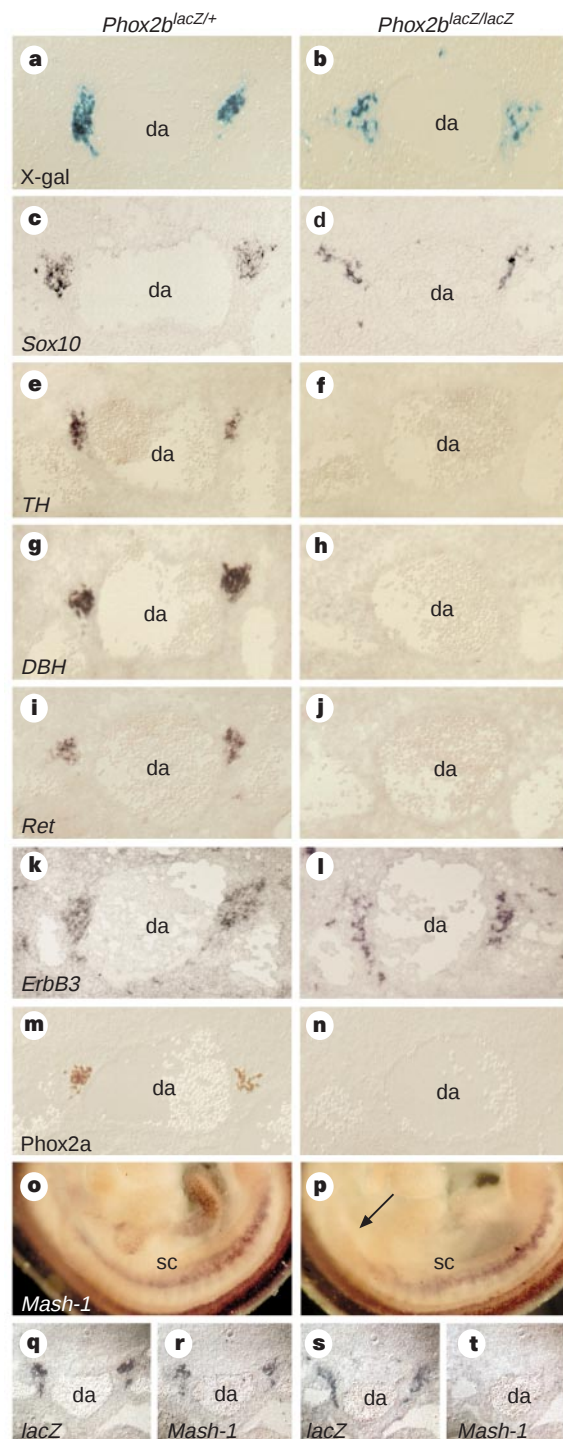


Figure 4 Altered gene expression in the early sympathetic chain of *Phox2b^{LacZ/LacZ}* embryos. **a–n**, Transverse sections through the sympathetic chain of heterozygous (**a, c, e, g, i, k, m**) and homozygous (**b, d, f, h, j, l, n**) E10.5 *Phox2b^{LacZ}* embryos at equivalent rostro-caudal levels. **a, b**, X-gal stain showing that sympathoblasts are present in the mutant embryos (**b**), albeit in reduced numbers. **c, d**, *In situ* hybridization with a *Sox10* probe showing the presence of neural-crest-derived progenitors. **e–n**, *In situ* hybridization with *TH* (**e, f**), *DBH* (**g, h**), *Ret* (**i, j**) and *ErbB3* (**k, l**) probes and immunohistochemistry with *Phox2a* antibody (**m, n**), showing loss of all these markers except *ErbB3* in *Phox2b^{LacZ/LacZ}* ganglia. **o–p**, Whole-mount *in situ* hybridization with a *Mash1* probe in heterozygous (**o**) and homozygous (**p**) *Phox2b^{LacZ}* embryos. Rostral is to the left. *Mash1* expression is already fainter in the rostral part of the mutant sympathetic chain which develops earlier (arrow in **p**). **q–t**, *In situ* hybridization with *LacZ* (**q, s**) and *Mash1* (**r, t**) probes on adjacent transverse sections of heterozygous (**q, r**) and homozygous (**s, t**) embryos at E11.5. *Mash1* expression is completely abolished in the rostral half of the chain. da: Dorsal aorta; sc: sympathetic chain.

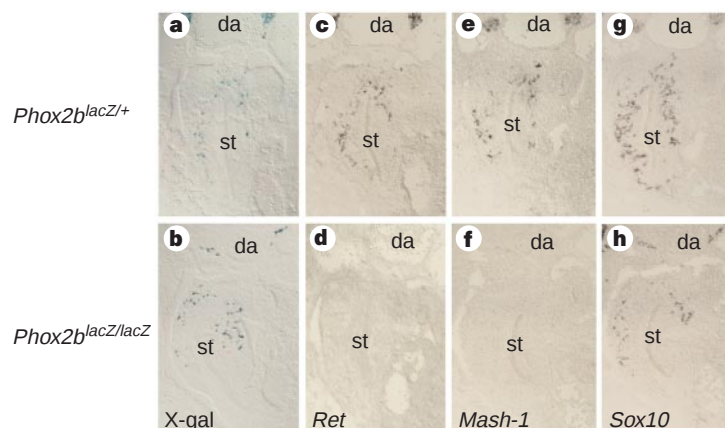


Figure 5 Altered gene expression in foregut enteric-neuron precursors at E10.5. **a, b**, X-gal stain on transverse sections through the stomach of heterozygous (**a**) and homozygous (**b**) mutant embryos. In homozygotes, enteric-neuron precursors are present, although reduced in number. **c–h**, *In situ* hybridization with

Ret (**c, d**), *Mash1* (**e, f**) and *Sox10* (**g, h**) probes on adjacent transverse sections through the anterior stomach of heterozygous and homozygous mutant embryos. Only *Sox10* is expressed in the mutant. st: stomach.

apoptotic nuclei in the wall of the foregut of heterozygous animals (Fig. 3f), but there were many in homozygous mutants (Fig. 3h). Hence, the foregut contingent of the enteric nervous system degenerates by apoptosis between E10.5 and E13.5, and migration of enteric neural precursors towards the mid- and hindgut is arrested at the stomach.

We then searched for differentiation defects in the early anlagen of the sympathetic and enteric nervous systems that might reveal what part of the autonomic differentiation program is controlled by *Phox2b*. *Phox2* genes may be determinants of the noradrenergic phenotype^{14–16}. But as the expression of tyrosine hydroxylase (TH) and dopamine- β -hydroxylase (DBH) is intact in the sympathetic ganglia of *Phox2a* mutants¹⁶, they may depend on *Phox2b* in these cell types. Indeed, *TH* and *DBH* expression was absent in all sympathetic and enteric progenitors and in the adrenal medulla of *Phox2b*^{LacZ/LacZ} animals (Fig. 4e–h; and data not shown). In addition, no DBH transcripts were found in the central nervous system, where the anlage of the locus coeruleus, the main noradrenergic centre of the brain, normally expresses them at this stage (data not shown). Thus, *Phox2b* is an essential determinant of the vertebrate noradrenergic phenotype. The lack of noradrenaline synthesis in *Phox2b*^{LacZ/LacZ} mutants might explain their embryonic lethality, as DBH is essential for development beyond mid-gestation²².

Glial-derived neurotrophic factor (GDNF) and its receptor subunits have been implicated in the development of most of the enteric nervous system, as well as that of the SCG^{6–12}. There is a regulatory link between *Phox2a* and *Ret* in the cranial sensory ganglia¹⁶. Moreover, the defect in the enteric nervous system of *Phox2b*^{LacZ/LacZ} mice at E10.5 is similar to that of *Ret*^{–/–} mutants. We therefore monitored *Ret*, which is normally expressed in early aggregating sympathoblasts^{3,10} and in migrating enteric neuroblasts²¹; there was no *Ret* expression in the sympathetic and enteric anlagen of mutant embryos (Figs 4i, j and 5a–d). Regulation of *Ret* by *Phox2b* could account for the failure of the post-oesophageal enteric nervous system to develop and the absence of the SCG in *Phox2b* mutants. However, other effector targets of *Phox2b* must be responsible, either alone or redundantly with *Ret*, for the survival and differentiation of sympathetic ganglia caudal to the SCG and of the foregut neurons, which are spared by the *Ret* mutation¹⁰. We also examined the expression of the neuregulin receptor ErbB3, which is necessary for sympathetic ganglion development¹³. ErbB3 was expressed in the ganglionic anlagen (Fig. 4k, l) and by the β -Gal-expressing enteric precursors (not shown) in the *Phox2b*^{LacZ/LacZ} embryos, in line with the fact that its expression, on emigrating neural-crest cells¹³, precedes that of *Phox2b*.

We investigated possible regulatory relationships between *Phox2b* and the basic-helix–loop–helix transcription factor *Mash1*, a mammalian homologue of the *Drosophila* proneural genes²³. In *Mash1*^{–/–} embryos, most sympathetic ganglia, a subset of enteric ganglia and the parasympathetic ganglia are missing^{3–5}. *Mash1* controls *Phox2a* (but not *Phox2b*) expression in sympathetic and parasympathetic ganglionic anlagen⁵, and forced expression of *Mash1* induces *Phox2a* in neural-crest stem cells²⁴. In E10.5 *Phox2b*^{LacZ/LacZ} mice, *Mash1* was expressed in the sympathetic ganglionic anlagen (Fig. 4o, p). However, *Phox2a* was not expressed (Fig. 4m, n), in line with previous indications that *Phox2a* and *Phox2b* regulate each other¹⁵. Therefore, *Phox2b* and *Mash1* are activated independently in sympathoblasts and are both necessary for *Phox2a* expression. As *Phox2a* is not needed for the formation and noradrenergic differentiation of sympathetic ganglia¹⁶, the functional significance of this dual regulatory requirement is unknown. At E10.5, *Mash1* was not expressed in the most rostral part of the sympathetic chain (Fig. 4o, p) where sympathoblasts are still present (Fig. 3a, b), suggesting an early down-regulation of *Mash1*. We confirmed this by showing that *Mash1* expression is shut down in the mutants at more caudal levels by E11.5 (Fig. 4q–t). In the enteric precursors, we could never detect *Mash1* transcripts (Fig. 5e, f). The rapid time course of degeneration of the mutant enteric nervous system makes it difficult to tell whether the lack of *Mash1* expression there is due to a failure of its induction or of its maintenance. These data, combined with earlier results, demonstrate the complexity of the regulatory relationship between *Phox2* genes and *Mash1* throughout the autonomic nervous system: *Mash1* regulates the expression of *Phox2a*^{5,24} but not of *Phox2b*⁵, which is itself necessary for the maintenance but not for the induction of *Mash1* expression.

We also examined the fourth division of the peripheral nervous system that expresses the *Phox2* genes: the epibranchial placode-derived visceral sensory ganglia of the VIIth, IXth and Xth cranial nerves, whose precursors start expressing *Phox2a* in the placodes^{14,25} and *Phox2b* as they delaminate and aggregate to form the ganglionic anlagen¹⁵. These ganglia are atrophic in *Phox2b*^{LacZ/LacZ} mid-gestation embryos (Fig. 6a–d), as they are in *Phox2a* mutants¹⁶. It is likely that their atrophy is caused by increased apoptotic cell death, detected at ~E11.5 (Fig. 6e, f). The ganglionic anlagen appear morphologically normal up to E10.5 (Fig. 6g, j). However, their expression of *Ret* is considerably reduced (Fig. 6k, l), and the transient expression of DBH in the ganglion cells is abolished (Fig. 6m, n). These data, combined with the very similar ganglionic phenotype of *Phox2a*^{–/–} mutants¹⁶ and our observation that *Phox2b* is controlled by *Phox2a* in cranial ganglia¹⁵, indicate that, in those structures, *Phox2b* is a downstream effector of *Phox2a*. We also

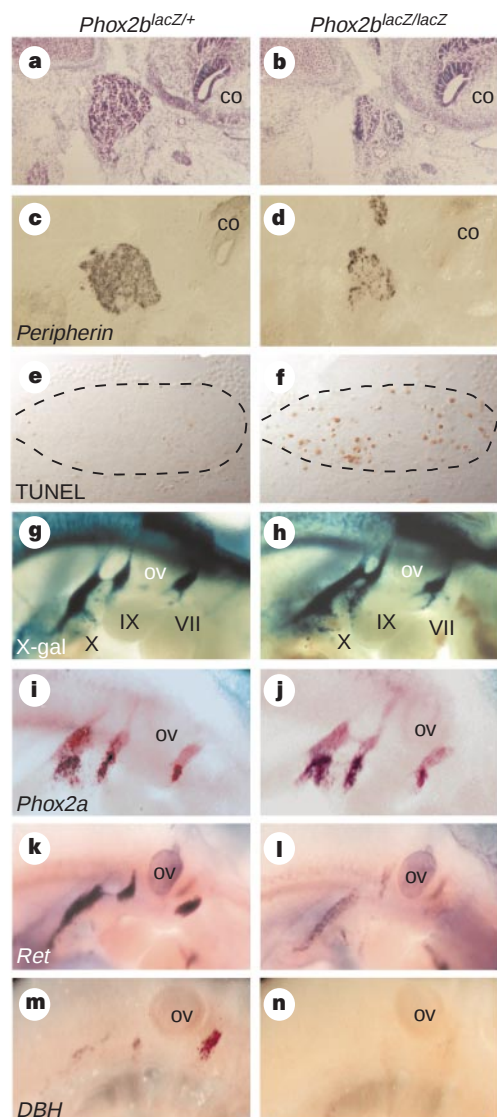


Figure 6 Formation and atrophy of placode-derived cranial ganglia VII, IX and X in *Phox2b^{lacZ/+}* mice. **a–d**, Nissl stain (**a, b**) and *in situ* hybridization with a *peripherin* probe (**c, d**) on parasagittal sections of E13.5 heterozygous (**a, c**) and homozygous mutant (**b, d**) embryos showing the atrophy of the nodose-petrose complex. **e, f**, Increase in apoptosis²⁰ in the anlage of the nodose-petrose complex at E11.5 in homozygous (**f**) compared with heterozygous animals (**e**). Dashed lines show the limits of the ganglion anlage as revealed by LacZ expression on adjacent sections. **g–j**, X-gal stain (**g, h**) and *Phox2a* *in situ* hybridization (**i, j**) on whole mounts of heterozygous (**g, i**) and homozygous (**h, j**) mutant E10.5 embryos showing normal morphology of the VIIth, IXth and Xth cranial ganglia in mutants. **k–n**, Whole-mount *in situ* hybridization with a *Ret* probe at E10.5 (**k, l**) and with a *DBH* probe at E9.5 (**m, n**) in heterozygotes (**k, m**) and homozygote (**l, n**) mutants. *Ret* expression is much reduced in mutants, and the expression of *DBH* is undetectable. co: Cochlea; ov: otic vesicle.

observed defects in the differentiation of branchiomotor neurons in the rhombencephalon of *Phox2b^{-/-}* mice (A.P., J.-F.B. and C.G., unpublished results).

These results reveal an unexpected ontogenetic similarity between the sympathetic, adrenal, parasympathetic and enteric derivatives of the neural crest, as well as placode-derived visceral sensory neurons, in the form of a requirement for the homeobox gene *Phox2b*. Therefore, beyond their developmental, anatomical and functional idiosyncrasies, the inclusion of all these classes of neurons in the physiologically coherent entity of the 'autonomic nervous system', or, more appropriately, the 'visceral neurons,

efferent and afferent'²⁶ is paralleled by a common developmental pathway. It remains to determine how far upstream and downstream of *Phox2b* this ontogenetic unity holds true. □

Methods

Targeting vector. Mouse *Phox2b* genomic clones were isolated from a genomic 129/Sv phage library. Details of the construction of the targeting vector are available upon request.

Morphology. Nissl staining was performed as described⁵. β -Galactosidase histochemistry was done according to ref. 27. Antisense RNA probes for *TH*, *DBH*, *peripherin*, *Ret*, *Sox10*, *Mash1*, *ErbB3* and *LacZ* were labelled using a DIG RNA labelling kit (Boehringer Mannheim). *In situ* hybridization with these probes, immunohistochemistry with anti-Phox2a antibodies and immunofluorescence with anti-neurofilament heavy-chain serum (Sigma) were done as described^{14,15}. Neurons undergoing apoptotic cell death in the sympathetic chain and in the foregut were detected by the TUNEL²⁰ method using the Apoptag detection kit (Oncor).

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Clathrin self-assembly is mediated by a tandemly repeated superhelix

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Clathrin is a triskelion-shaped cytoplasmic protein that polymerizes into a polyhedral lattice on intracellular membranes to form protein-coated membrane vesicles. Lattice formation induces the sorting of membrane proteins during endocytosis and organelle biogenesis by interacting with membrane-associated adaptor molecules¹. The clathrin triskelion is a trimer of heavy-chain subunits (1,675 residues), each binding a single light-chain subunit, in the hub domain (residues 1,074–1,675). Light chains negatively modulate polymerization so that intracellular clathrin assembly is adaptor-dependent². Here we report the atomic structure, to 2.6 Å resolution, of hub residues 1,210–1,516 involved in mediating spontaneous clathrin heavy-chain polymerization and light-chain association^{3,4}. The hub fragment folds into an elongated coil of α -helices, and alignment analyses reveal a 145-residue motif that is repeated seven times along the filamentous leg and appears in other proteins involved in vacuolar protein sorting. The resulting model provides a three-dimensional framework for understanding clathrin heavy-chain self-assembly, light-chain binding and trimerization.

Clathrin heavy-chain residues 1,210–1,516 are centrally located within the proximal segment of the triskelion leg which, along with the trimerization domain and carboxy terminus, forms the triskelion hub (Fig. 1a). When clathrin polymerizes, these residues comprise the primary interaction site between proximal leg domains in a clathrin lattice (Fig. 1b)^{3–5}. The crystal structure of residues 1,210–1,516 reveals an elongated right-handed superhelix coil of short α -helices (Fig. 2a). Individual 'helix-turn-helix-loop' or helix hairpin units comprise the canonical repeat and stack along the superhelix axis to form a single extended domain (Fig. 2b). The model's rigidity is attributed primarily to typical interdigitated packing of crossing antiparallel α -helices⁶ and to a continuous hydrophobic core maintained by aromatic and bulky hydrophobic amino-acid side chains. The superhelix resembles a flattened rod 115 Å long and with a 24 × 28 Å oval cross-section that presents two major faces consisting of parallel α -helices spanning the length. The parallel α -helices are tilted -70° to -80° relative to the superhelix axis and are 10–15 Å apart in pitch.

Right-handed superhelices of α -helices have been reported in domains of various enzymes^{7–9} and signalling proteins^{10–13}. In all examples, this fold serves as a scaffold for the presentation of α -helices in protein–protein interactions. The armadillo repeats of β -catenin¹¹ and karyopherin¹² are generated from three-helix motifs, whereas superhelices in clathrin and other examples arise from two-helix repeats. Clathrin represents a unique member of the α/α -superhelix structural category of proteins¹⁴ because its superhelix

axis propagates along a straight, rather than curved, path and because its faces of parallel α -helices exhibit little or no overall twist or spiralling (Fig. 2). These distinctive aspects are likely to have a functional impact on assembly.

The canonical hairpin repeat of the clathrin superhelix resembles a tetratricopeptide repeat (TPR)¹⁵, but is shorter and lacks the characteristic spacing of the hydrophobic residues in TPRs. Within the clathrin heavy chain itself, the helix hairpins of the proximal leg show a striking resemblance to hairpin motifs identified in the crystal structure of the amino-terminal domain¹⁶ (Fig. 1a). This β -structure-rich region terminates in an irregular right-handed twisting of ten successive hairpins, termed an α -zigzag linker¹⁶. Hairpins of the superhelical leg region and the α -zigzag linker are approximately equal in average length, but the lengths of individual helices vary significantly more within the α -zigzag region than within the proximal leg fragment. The α -zigzag may represent a flexible, irregularly folded variant of the α/α superhelix typified in the proximal leg. Relaxation of helix and hairpin length constraints permits contrasting structural forms and allows different roles for the linker versus the lattice component represented by the rest of the triskelion leg.

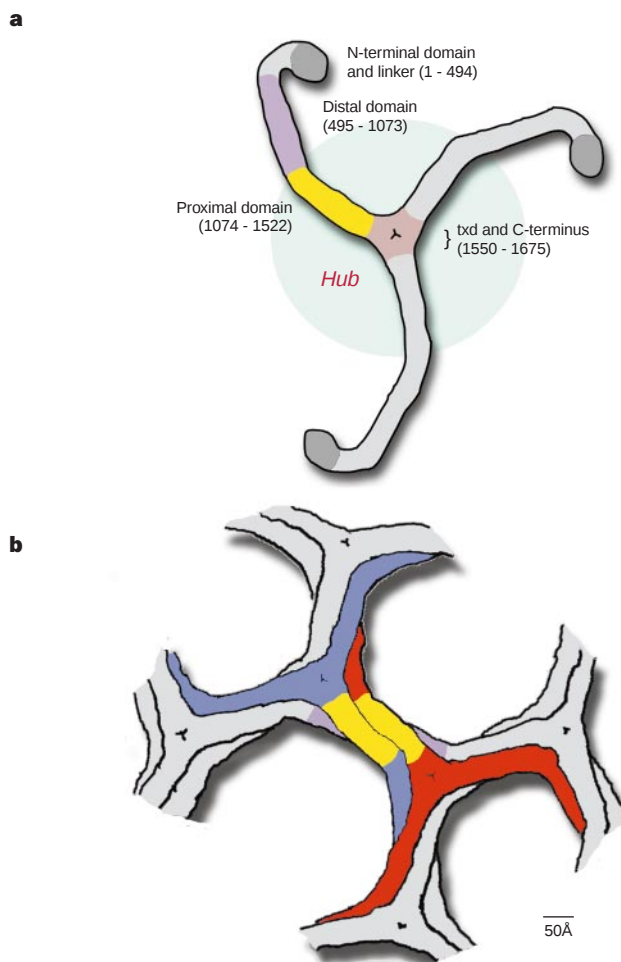


Figure 1 Organization of the clathrin polyhedron and triskelion. **a**, Clathrin triskelion with heavy-chain domains and their approximate sequence boundaries indicated. Dark grey, globular N-terminal domain; purple, distal domain; yellow, proximal domain; pink, trimerization domain (TXD). The light blue circle encompasses the hub region (1,074–1,675). **b**, Portion of the clathrin polyhedron lattice, with a triskelion centred at each vertex, after ref. 5. Two nearest-neighbour, associating triskelions are coloured red and blue. Yellow indicates the portion of the proximal domain determined in the crystal structure. Each lattice edge is comprised of antiparallel-associated proximal domains and antiparallel-associated distal domains (purple) lying underneath.

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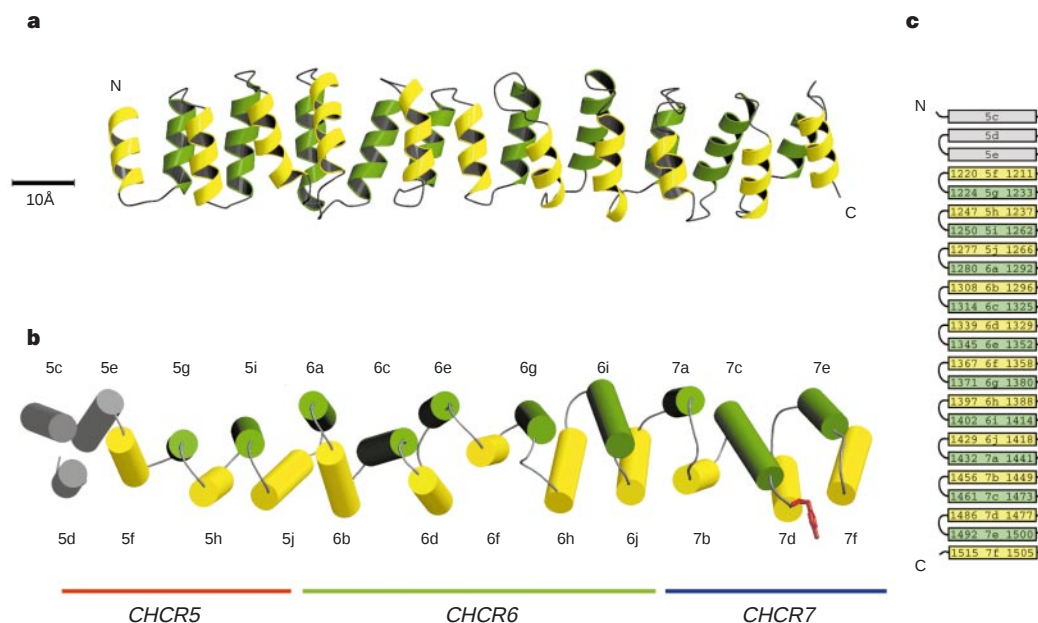


Figure 2 The crystal structure of clathrin heavy-chain residues 1,210–1,516. The two vertically aligned views are related by 90° rotation around the long axis of the protein. Images prepared with BOBSCRIPT²⁸. **a**, Ribbon diagram, viewed on the helix face. Canonical helix hairpin unit is an N-terminal green helix joined to C-terminal yellow helix. Helices of each colour form a separate face or layer of parallel α -helices. Hairpin turns form an 'edge' along the top. **b**, Cylinder representation, viewed on the hairpin edge, showing the relative orientation of individual helices, named as labelled. Side chain of Tyr 1,477 (red) is indicated. Grey cylinders represent helices 5c, 5d and 5e, identified in the electron-density map, but whose side chains could not be assigned. Red, green and blue bars delineate regions of CHCR5, CHCR6 and CHCR7, respectively. **c**, Topology diagram, indicating sequence boundaries of helices, labelled as in **b**.

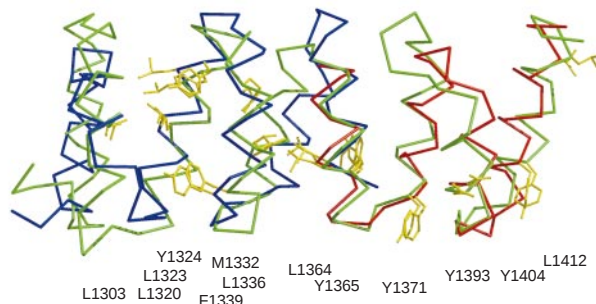


Figure 3 Optimized superposition of C α traces from CHCR5, CHCR6 and CHCR7 regions in the clathrin proximal leg, determined with LSQMAN²⁷. CHCR6 (green), residues 1,274–1,416, comprise a complete 10-helix motif. CHCR5 (red), 1,210–1,270, and CHCR7 (blue), 1,423–1,516, superposed on CHCR6 with r.m.s. deviations

of 1.90 and 1.95 Å, respectively. Overlapping identical or similar side-chains resulting from the C α -based structural alignment are highlighted in yellow and labelled according to their position in CHCR6. Note that two full CHCRs are represented in the solved structure.

Sequence and coordinate-alignment analysis of the hub-fragment crystal structure found a clathrin heavy-chain repeat (CHCR) motif in the distal and proximal leg regions. Seven complete repeats (designated CHCR1–CHCR7) were found over the triskelion leg (residues 537–1566). One complete repeat, CHCR6, and the C-terminal and N-terminal halves of adjoining repeats (CHCR5 and CHCR7) are in the atomic model of residues 1,210–1,516 (Fig. 2b). The structural motif of five helix hairpins, rather than a single helix hairpin, was found by comparing C α superpositions between all combinations of grouped α -helices within the model. Groups of as few as three α -helices superposed best when separated by a length of 10 helices (5 hairpins), or approximately 145 residues. The optimized C α alignment of CHCR5, CHCR6 and CHCR7 results in root mean square (r.m.s.) deviations of 1.9 Å and produces 12 amino-acid side-chain coincidences, mainly at positions of bulky hydrophobic and aromatic residues involved in helix–helix packing (Fig. 3). Further analysis of clathrin heavy-chain sequences by the generalized profile method¹⁷ yielded a structure-based sequence alignment of the seven repeated motifs (Fig. 4a).

By sequence analysis, we also identified clathrin repeat motifs in non-clathrin yeast proteins previously noted as homologues¹⁸, as well as in their homologues from other eukaryotes (Fig. 4b). These proteins are implicated in vacuolar maintenance and protein sorting¹⁸. Among these proteins, Vps41/Vam2 and Vps39/Vam6 interact with each other and form part of a large complex associated with the vacuolar membrane¹⁹. The clathrin CHCR motifs in these proteins could mediate protein–protein interactions, or possibly represent clathrin-binding domains, or perform clathrin-like functions.

A helix hairpin motif has been proposed to exist throughout the clathrin leg¹⁶. The crystal structure of the proximal leg, together with the organization of seven CHCR repeats (residues 537–1,566), confirms this, and indicates further that the filamentous clathrin leg is formed by a continuous α/α -superhelix fold, with helix hairpins more tightly and regularly packed than in the linker region of the leg¹⁶. Between the linker and CHCR1, residues 494–536 conform to a partial CHCR motif. In addition, as CHCR7 (1,423–1,566) overlaps with the trimerization domain, the super-

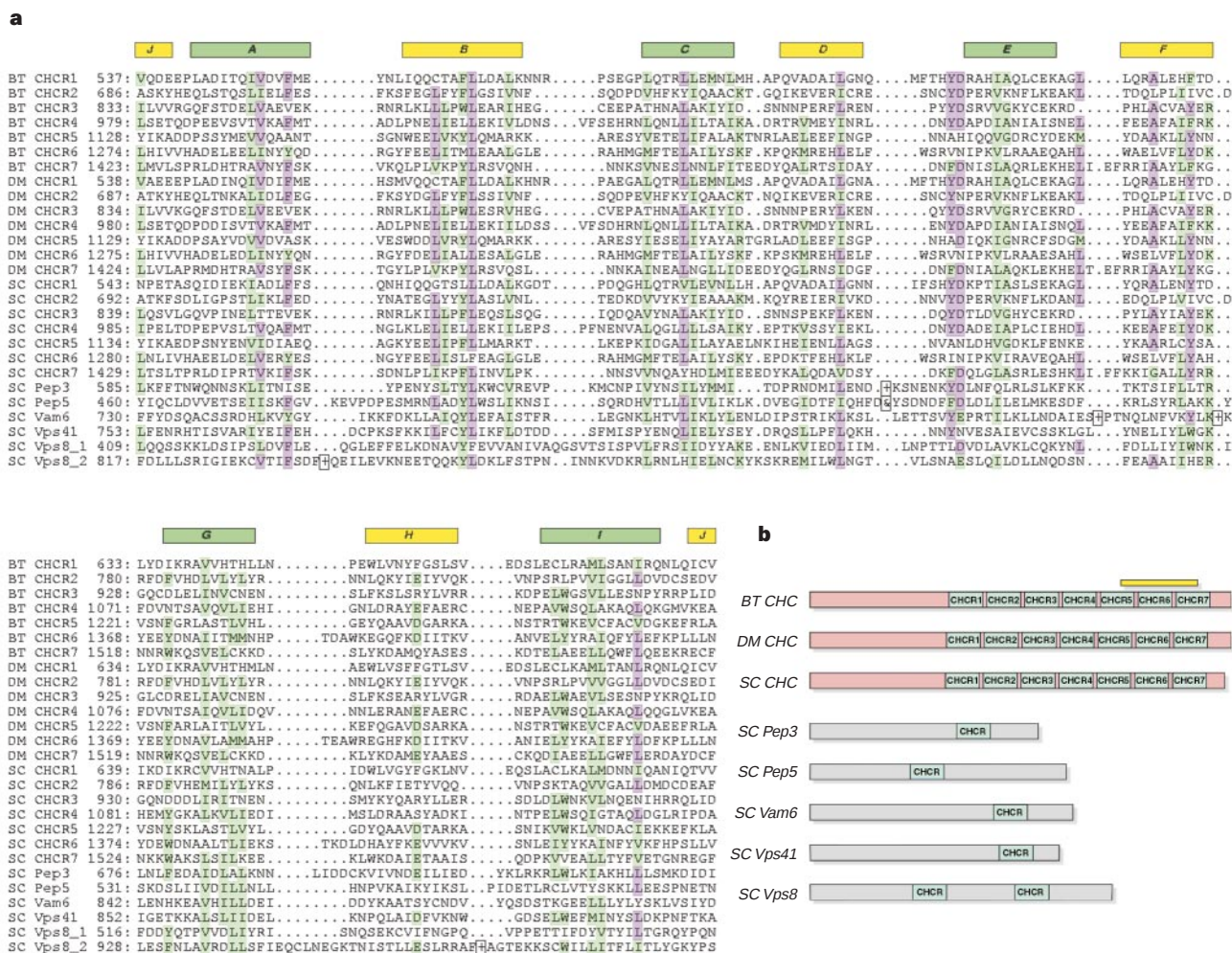


Figure 4 Sequence analysis of clathrin heavy-chain and related sequences. **a**, Multiple sequence alignment of the clathrin heavy chains and related sequences. All sequences match profile with probabilities of error <0.01. Magenta, 50% or more of the aligned residues are identical. Green, 50% or more of the aligned residues belong to one similarity group. +, positions of 5-residue insertion; &1, position of 20-residue insertion. Clathrin heavy-chain repeats are labelled CHC1 to CHC7. Clathrin species shown: BT, *Bos taurus* (bovine); DM, *Drosophila melanogaster* (fruitfly); and SC, *Saccharomyces cerevisiae* (yeast). Non-clathrin

proteins form *S. cerevisiae*: Pep3, Pep5, Vam6, Vps41 and Vps8. Sequences fitting the alignment profile but not shown include clathrin heavy chains of dictyostelium, yeast *S. pombe* and the human muscle clathrin isoform CHC22, plus homologues of the represented non-clathrin proteins. **b**, Schematic domain organization of represented clathrin (pink) and non-clathrin (grey) proteins containing clathrin repeats (green). Yellow bar indicates the region of solved crystal structure.

helix fold probably propagates into the centre of the triskelion vertex, thereby allowing interactions between opposing stacked helix hairpins to mediate trimerization.

The structure reported here confirms that the filamentous portion of the clathrin triskelion leg is primarily α -helical, as indicated by its circular dichroism spectrum²⁰, and also shows that the helices orient perpendicular to the triskelion leg as they distribute linearly from the linker region to the C terminus. This corrects our previous hypothesis that α -helices in the clathrin leg might extend longitudinally and fold back on themselves³. In addition, the superhelix fold indicates that association of the regulatory clathrin light-chain subunit is unlikely to be mediated by an extended coiled-coil interaction, as previously proposed^{3,21}.

Possible sites for light-chain binding are indicated by the surface features of the crystal structure. One prominent groove is formed along the exposed surface of helices 7a, 7c and 7e (Fig. 2b). This region is distinctly basic compared to the rest of the structure (Fig. 5a), and could accommodate at least a portion of the highly acidic light chain. Near the entrance of the groove is Tyr 1477 (Figs 2b and 5), a site of phosphorylation by Src kinase during ligand-induced endocytosis of the receptor for epidermal growth

factor, which has a proposed regulatory influence in clathrin recruitment²². Natural substitutions in a clathrin heavy-chain homologue, encoded on chromosome 22 (CHC22) and expressed primarily in skeletal muscle, also implicate this groove in binding the light chain. CHC22 fails to bind light chains (S.H.L., E. Chen and F.M.B., unpublished results), despite having 84% sequence identity with the conventional clathrin heavy chain. A significant cluster of CHC22 substitutions lies along this groove, including three substitutions that remove basic charge (Fig. 5b).

Finally, the crystal structure indicates how proximal legs might associate in lattice assembly. The model at 21 Å resolution of the clathrin polyhedron⁵ reveals that the physical association of two proximal legs requires lengthwise interaction of common surfaces (Fig. 1b). We suggest that proximal regions associate via helix-face/helix-face contacts, facilitated by the complementary packing of helix ridges and grooves. This would allow large hydrophobic patches along open grooves (Fig. 5c) to become buried during lattice formation. Solvent-exposed histidines at or near turns (Fig. 5c) could additionally stabilize the face-to-face association. Histidines have been implicated in clathrin assembly at pHs lower than physiological². The juxtaposition of paired histidine residues

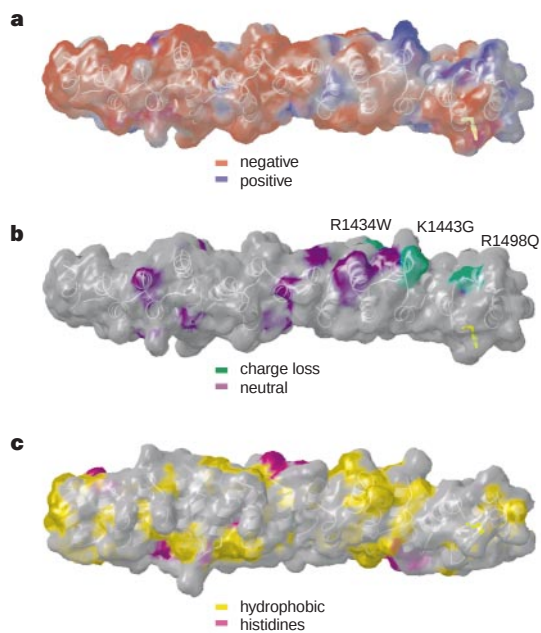


Figure 5 Surface representations of clathrin heavy chain, residues 1210–1516. Mappings of features onto the solvent-accessible surface were prepared with GRASP²⁹ and rendered using RASTER3D³⁰. Side chain of Tyr 1,477 (yellow–lime) is indicated in all panels. **a**, Surface electrostatic potential, with model oriented as in Fig. 2b. Red, negative charge; blue, positive charge. **b**, Distribution of muscle clathrin substitutions (purple/green), same view as **a**. Green substitutions (labelled) produce loss of side-chain charge. Blue represents neutral substitutions. **c**, Distribution of exposed hydrophobic regions (yellow) and exposed histidines (magenta). Helix-face view, with Tyr 1,477 towards viewer, rotated approximately 90° relative to **a**. Histidines of proximal leg, highly conserved among clathrins, tends to be exposed and positioned near loops and turns.

also suggests a role for metal binding in regulating clathrin assembly, consistent with the observation that 2–5 mM ZnCl₂ inhibits assembly (J.A.Y. and F.M.B., unpublished results). The structure reported here establishes the fundamental self-assembling motif for clathrin polymerization and potentially for other protein complexes involved in vacuolar protein sorting. □

Methods

Expression, purification and crystallization. The plasmid encoding the bovine heavy-chain fragment 1,074–1,522 with an N-terminal polyhistidine tag was constructed as described⁴. The selenomethionine-substituted heavy-chain fragment was produced as described²³. After induction, the cells were lysed by sonication in cold lysis buffer at pH 7.9 with protease inhibitors. The cleared bacterial lysate was passed through a nickel-affinity column (Pharmacia), then passed through a POROS 20 HQ anion-exchange column (Perseptive Biosystems) equilibrated with 10 mM ethanolamine buffer, pH 9. The protein was eluted with a 2–3% gradient of 10 mM ethanolamine, 0.25 M NaCl at pH 9. The fractions were pooled, concentrated and then passed through a Superose-6 column (Pharmacia) equilibrated in 10 mM HEPES, 3 mM TCEP (Pierce) at pH 7.9. We confirmed the 13 selenomethionine substitutions by electrospray mass-spectroscopy. The purified protein was concentrated and crystallized in hanging drop by vapour diffusion against reservoir containing 250 mM sodium formate, 100 mM Tris-HCl, pH 8. Crystals grew in the tetragonal space group *I*4122 (*a* = 145.5, *b* = 145.5, *c* = 166.6), with one molecule in the asymmetric unit.

Data collection. The structure was solved by multiwavelength anomalous dispersion (MAD). A 4-wavelength data set was collected from a single crystal of selenomethionine-substituted clathrin heavy-chain fragment on beamline 5.0.2 at the Advanced Light Source, Lawrence Berkeley National Laboratory. The crystal was maintained at 100 K using an Oxford Cryostream, and data

were collected in 1° oscillations using a 188 mm × 188 mm Quantum-4 CCD detector system from Area Detector Systems Corp. Two wavelengths, λ₂ and λ₃, were at the inflection point and peak of the K-edge of selenium at 0.9797 and 0.9794 Å, respectively, while the two others, λ₁ and λ₄, were low and high-energy remote wavelengths of 1.000 and 0.9686 Å. Each wavelength was collected in a single sweep. Data were integrated and scaled using the Denzo and Scalepack²⁴ programs.

Phasing and refinement. The MAD data were initially phased by treating the low remote wavelength (λ₁) as the 'native' or reference wavelength and the others as 'derivative' using a Bayesian approach in which diffraction at four wavelengths was reduced to single isomorphous and anomalous scattering contributions²⁵. Eight of the possible 13 selenium sites were found from the automated Patterson search in SOLVE (<http://www.solve.lanl.gov>). The same eight sites were also identified using the direct-methods algorithm of SnB2 (<http://www.hwi.buffalo.edu/SnB>) with the λ₃ dataset alone. The remaining selenium atoms occur in the disordered N-terminal region of the protein and were never visualized. We refined the selenium model at 2.7 Å further using the maximum likelihood algorithm implemented in SHARP (<http://lagrange.mrc-lmb.cam.ac.uk/sharp/SharpHome.phtml>). Experimental phases (α) were estimated at 2.6 Å resolution, giving final figures of merit of 0.65 and 0.44 for acentric and centric reflections, respectively. The phases calculated in SHARP were improved using iterative solvent flattening (solvent content of 52%). The resulting |F_{obs}|/α Fourier map showed good contrast between solvent and protein regions, with a right-handed superhelix of α-helices clearly visible, allowing us to build an initial model for 250 of the 450 protein residues.

Experimental phases produced a high-quality electron-density map that was improved by density modification and phase combination. Refinement was carried out using the λ₁ dataset from 7.0–2.6 Å in the program CNS²⁶. Bijvoet pairs were kept separate throughout, and the anomalous scattering terms of selenium were included in the calculation of structure factors. Refinement proceeded by rounds of positional, grouped B factor and simulated annealing interspersed with model building with reference to 2F_o – F_c and F_o – F_c maps. Near the end of refinement, a bulk-solvent correction was applied, and the structural model refined against all the data (30.0–2.6 Å) with an *R*-factor of 24.7% and a free *R*-factor of 27.8% at 2.6 Å resolution. The electron density for the polypeptide backbone was everywhere continuous at 1.3σ in a 2F_o – F_c difference Fourier synthesis.

The final model contains no unfavourable φ, ψ combinations and main-chain and side-chain structural parameters consistently better than those expected at 2.6 Å resolution (overall G, 0.27; r.m.s.d. of bonds, 0.010; r.m.s.d. of angles, 1.34). The average atomic *B*-factor is 46.5 Å².

Structural and sequence analysis. Determination and comparison of Cα trace superpositions were carried out using LSQMAN²⁷ and yielded coordinate alignments between portions of the structural model. We constructed a sequence profile from structural alignments within the crystal structure and refined it in an iterative fashion¹⁷. The profile was run against the complete protein database, and regions in proteins that match to the profile with high significance (probability of error <0.01) were identified. Newly found matches were then incorporated into the alignment and a refined profile was constructed and again run against the protein database.

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A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria

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The virulence properties of many pathogenic bacteria are due to proteins encoded by large gene clusters called pathogenicity islands^{1,2}, which are found in a variety of human pathogens including *Escherichia coli*, *Salmonella*, *Shigella*, *Yersinia*, *Helicobacter pylori*, *Vibrio cholerae*, and animal and plant pathogens such as *Dichelobacter nodosus* and *Pseudomonas syringae*^{1–3}. Although the presence of pathogenicity islands is a prerequisite for many bacterial diseases, little is known about their origins or mechanism of transfer into the bacterium. The bacterial agent of epidemic cholera, *Vibrio cholerae*, contains a bacteriophage known as cholera-toxin phage (CTX Φ)⁴, which encodes the cholera toxin, and a large pathogenicity island called the VPI (for *V. cholerae* pathogenicity island)⁵ which itself encodes a toxin-

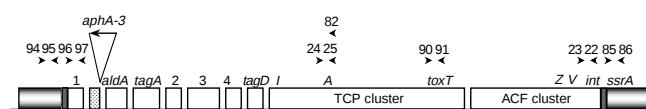


Figure 1 Diagram of the VPI. Arrows above genes indicate the PCR primers used and are identified by the number of the KAR primer series. Black bars, regions targeted for PCR; grey bars, common chromosomal flanking DNA; checked regions at each end of VPI denote *att* sites; dotted region (left) in the VPI represents the defective transposase (*xtn*). The *aphA-3* gene encoding Km resistance is inserted into a *Bam*HI site between *xtn* and *aldA*.

coregulated pilus that functions as a colonization factor⁶ and as a CTX Φ receptor⁴. We have now identified the VPI pathogenicity island as the genome of another filamentous bacteriophage, VPI Φ . We show that VPI Φ is transferred between *V. cholerae* strains and provide evidence that the TcpA subunit of the toxin-coregulated type IV pilus is in fact a coat protein of VPI Φ . Our results are the first description of a phage that encodes a receptor for another phage and of a virus-virus interaction that is necessary for bacterial pathogenicity.

Cholera is an ancient and life-threatening epidemic disease that occurs worldwide^{7,8}. Virulent and epidemic strains of *V. cholerae* require two genetic elements to cause disease, CTX Φ ⁴ and VPI⁵. CTX Φ encodes the cholera toxin responsible for the severe secretory diarrhoea characteristic of the disease⁴. The VPI (Fig. 1) is required for the emergence of *V. cholerae* as it contains the toxin-coregulated pilus (TCP) gene cluster which encodes a type-IV pilus that functions both as an essential colonization factor^{6,9} and as a CTX Φ receptor⁴. VPI has many features of bacterial pathogenicity islands (PAIs): it is large (~40 kilobases), contains genes associated with virulence, regulation and mobility, is inserted into a single chromosomal site (*att* site) adjacent to a tRNA-like gene, and it has a different G + C content compared with the host chromosome^{4–6,10–13}.

We previously identified one *V. cholerae* strain (E9120) that has lost the VPI (ref. 5). This strain contains *ctx* genes, suggesting that it previously had the VPI to allow infection by CTX Φ , and has an altered *att* site, indicating that the VPI had been excised. As the VPI contains a transposase-like gene and a phage-like integrase gene^{5,13}, we investigated other VPI genes using BLAST¹⁴ to find ones whose predicted protein products share homology with phage or viral proteins. TagE has 30% identity and 55% similarity over 188 amino acids to Orf16 of *Staphylococcus aureus* bacteriophage Φ (Genbank accession number, AB009866); OrfZ has 26% identity and 41% similarity over 97 amino acids to an 'early' protein of rat cyto-

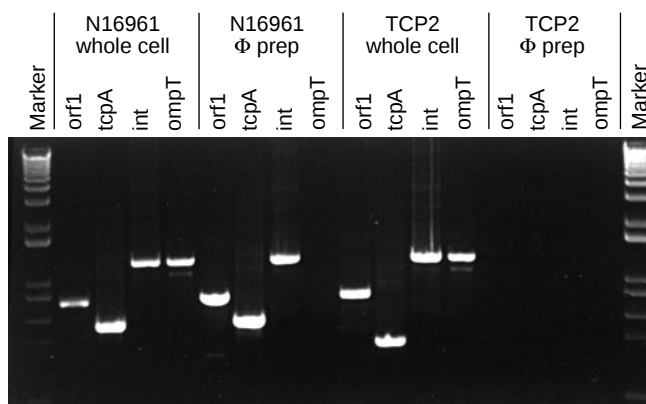


Figure 2 PCR products obtained from either whole-cell overnight cultures or cell-free phage (Φ) preparations of wild-type strain N16961 and TCP2. Note the presence of the VPI Φ genes *orf1*, *tcpA* and *int* in phage preparations of N16961 but not in the *tcpA* mutant strain TCP2. Markers are derived from a DNA ladder (0.1–12 kb; BRL).

Table 1 Detection of VPIΦ, CTXΦ and chromosomal genes

Strain	Sample	Detection of genes								
		VPIΦ			CTXΦ		Chromosomal			
		<i>orf1</i>	<i>tcpA</i>	<i>toxT</i>	<i>int</i>	<i>ctxB</i>	<i>zot</i>	flank VPI	<i>ompT</i>	<i>rfaD</i>
395	Whole cell	+	+	+	+	+	+	+	+	+
	Phage prep.	+	+	+	+	+	+	—	—	—
N16961	Whole cell	+	+	+	+	+	+	+	+	+
	Phage prep.	+	+	+	+	+	+	—	—	—
CVD110	Whole cell	+	+	+	+	+	—	+	+	+
	Phage prep.	+	+	+	+	—	—	—	—	—
TCP2	Whole cell	+	+	+	+	+	+	+	+	+
	Phage prep.	—	—	—	—	+	+	—	—	—
395-RF	Plasmid	+	+	+	+	+	+	—	—	—
N16961-RF	Plasmid	+	+	+	+	+	+	—	—	—
DK239	Whole cell	+	+	+	+	—	—	+	+	+
	Phage prep.	+	+	+	+	—	—	—	—	—

Genes were amplified by PCR from whole cell cultures, cell-free phage preparations and plasmid replicative-form preparations.

megalovirus (Genbank accession number, U62396); and OrfV has 32% identity and 47% similarity over 79 amino acids to the ‘enhancin’ protein of *Lymantria dispar* nucleopolyhedrovirus (Genbank accession number, AF019970). Even *TcpA* from the El Tor strain N16961 shows homology (31% identity, 48% similarity over 82 amino acids) to the product of a 769-amino-acid Orf from TT virus (Genbank accession number, AB011490). Taken together, these results indicated that the VPI could be the genome of a phage. We tested this idea by examining phage preparations of *V. cholerae* strains N16961 and 395 for genes at the ends and centre of the VPI. By using the polymerase chain reaction (PCR) and sequencing, we found *orf1*, *tcpA*, *toxT* and *int* genes in both phage preparations (Fig. 1, 2 and Table 1), but no PCR products were amplified when primers targeted regions immediately outside the VPI, nor for *ompT* (which encodes an outer membrane protein) (Fig. 2) or *rfaD* (involved in lipopolysaccharide synthesis), which are also outside the VPI. A phage preparation derived from a VPI-negative strain prepared under identical conditions was also negative for the chromosomal *ompT* and *rfaD*, as well as for *orf1*, *tcpA*, *toxT* and *int* genes. As expected, PCR analysis of the preparations identified *ctxB* and *zot* genes on CTXΦ. Finding VPI genes in phage preparations after treatment with DNase and RNase indicated that the DNA was protected, presumably by a protein coat, and that this element was probably a bacteriophage (designated VPIΦ).

Phage DNA from strains N16961 and 395 was sensitive to digestion with S1 nuclease (specific for single-stranded DNA) and S1-treated phage DNA gave no PCR products. The same phage DNA was resistant to digestion with restriction endonucleases specific for double-stranded DNA and yielded VPI PCR products after digestion. Southern-blot analysis on phage DNA from DK238 (see below) and CVD110 using *tcpA* forward and reverse primers revealed that the phage DNA hybridized only with the primer that could hybridize to the positive strand (Fig. 3). Thus, VPIΦ, like CTXΦ (ref. 4), contains positive, single-strand DNA as its genome. To determine whether VPIΦ has a plasmid replicative form (RF) in the cell, we analysed plasmid DNA from N16961 and 395 for VPI genes. RF preparations were sensitive to digestion with double-strand-specific *PvuII* (which has a site in *tcpA*) as *PvuII*-digested RF preparations failed to generate PCR fragments using *tcpA* primers (Fig. 4a), but were resistant to digestion with S1 nuclease and could still generate VPI PCR products after treatment with S1. PCR analysis revealed no chromosomal contamination as *ompT* and *rfaD* sequences were not amplified (Table 1). Southern-blot hybridization of RF preparations confirmed the presence of a VPIΦ replicative form. *HaeIII*-digested RF from DK238 and CVD110, as well as N16961 chromosomal DNA, hybridized with a *tcpA* PCR product probe, whereas only chromosomal DNA hybridized with an *ompT* probe (Fig. 4b, c). These results indicate that, like other

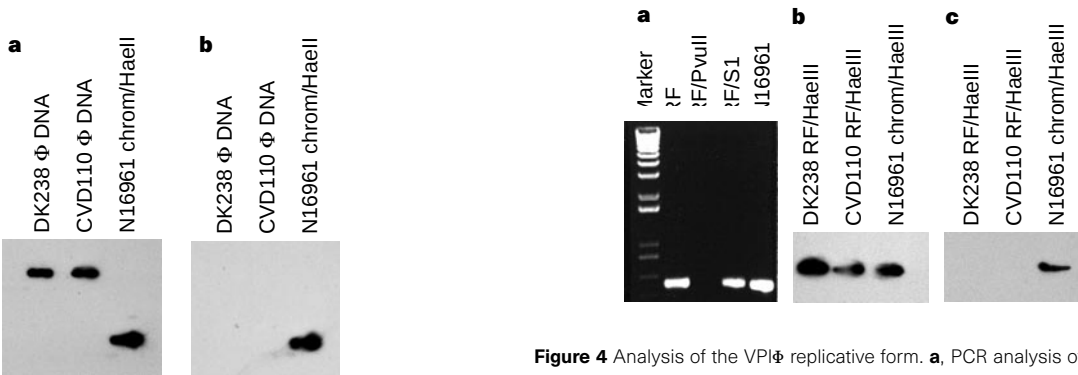


Figure 3 Southern blot of phage DNA preparations from DK238 and CVD110. **a**, Hybridization of the phage DNA with a single-stranded *tcpA* probe (KAR25) specific for the (+) strand. **b**, Lack of hybridization of phage DNA with a single-stranded *tcpA* probe (KAR24) specific for the (–) strand. Both probes hybridized to chromosomal DNA from *V. cholerae* N16961.

Figure 4 Analysis of the VPIΦ replicative form. **a**, PCR analysis of the RF using *tcpA* primers KAR24 and KAR25. Note that a *tcpA* product is not generated by using *PvuII*-digested RF as template, whereas RF treated with S1 nuclease (RF/S1) generates a PCR product. N16961 chromosome was used as a control template. **b**, Southern-blot hybridization of *HaeIII*-digested RF from DK238 and CVD110 probed with a *tcpA* PCR product; **c**, Southern-blot hybridization of *HaeIII*-digested RF probed with an *ompT* PCR product shows hybridization only to the N16961 chromosomal control.

filamentous phages, VPIΦ forms a double-stranded-DNA plasmid replicative form in the cell.

The VPIΦ genome of a spontaneously streptomycin-resistant (Str) strain (N16961) was marked with the *aphA-3* gene, which encodes resistance to kanamycin (Km) and neomycin (Neo), creating strain DK238. *aphA-3* was inserted between *aldA* and *xtn* (Fig. 1), a region encoding a putative non-functional transposase⁵. We used whole cells and cell-free phage preparations from donor DK238 to determine whether VPIΦ could be transferred into the non-toxicogenic VPI-negative strain DK236 (serogroup O10, nalidixic acid (Nal) resistant). After 1 hour of incubation at 37°C of 3×10^7 DK236 recipient cells with either 3×10^7 donor DK238 cells or phage preparations from 2×10^7 DK238 cells, cultures were plated onto agar containing Nal/Neo. The use of a Nal-sensitive donor and Nal-resistant recipient enabled us to identify Nal-resistant transductants that had acquired *aphA-3* from VPIΦ. With DK238 cells, we obtained 1.3×10^4 transductants, suggesting that, on average, about 0.04% of recipient cells were transduced under these conditions; with phage preparations from 2×10^7 donor cells, we obtained $> 3 \times 10^8$ transductants per 3×10^7 recipients, indicating that VPIΦ made by one donor cell gives rise to at least 15 transductants. The successful transfer of the neomycin-resistance marker with cell-free DNase-treated phage preparations indicated that transduction, rather than conjugation, was the mechanism of DNA transfer.

Transfer of VPIΦ from donor to recipient was confirmed for one transductant, DK239, by using O antigen agglutination, antibiotic-susceptibility testing, ribotyping, colony hybridization with *aphA-3* and *tcpA* probes, and PCR analysis of *aphA-3*, *tcpA*, *toxT* and *int* genes (Table 1). PCR on DK239 using primers for the right VPI

junction showed that the VPI had integrated into the same site as wild-type VPI-positive strains. PCR on phage preparations from DK239 indicates that, although DK239 produces VPIΦ, CTXΦ was not acquired (Table 1).

Not all *V. cholerae* strains could act as donors or recipients of VPIΦ. Although we detected VPIΦ particles in phage preparations of classical strain 395, no VPIΦ was transferred when a derivative of 395 (also marked by *aphA-3* in the VPI) was used as donor, suggesting that this strain is unable to transfer VPIΦ efficiently, or that different conditions are needed. With N16961 as donor, transfer occurred into the O10 strain DK236 but not into the non-toxicogenic VPI-negative strain DK237 (serogroup O1). As both DK236 and DK237 have a vacant *att* site⁵, these strains may differ in their ability to acquire VPIΦ, possibly explaining the limited number of toxicogenic serogroups. Epidemic and pandemic cholera is associated with O1 and O139 strains. Although we have shown that VPI⁺ non-O1/non-O139, potentially toxicogenic strains can be created *in vitro*, VPI⁺CT⁺ O1 strains may have an advantage *in vivo* or in the environment over VPI⁺CT⁺ non-O1 strains, explaining the predominance of O1 strains in epidemic and pandemic disease. Our results highlight the potential for serogroups of *V. cholerae* other than O1 and O139 to acquire the VPI and become pathogenic and even epidemic and pandemic strains.

Concentrated phage preparations of several VPI-positive *V. cholerae* strains were viewed under electron microscopy. Immunoelectron microscopy of preparations from strain 395 using rabbit anti-TcpA antibodies revealed many gold particles bound to parallel bundles of VPIΦ particles (Fig. 5a). El Tor strain CVD110, which has the *ctxA*, *zot*, *ace* and *orfU* genes deleted¹⁵, cannot produce CTXΦ or CTXΦ-encoded genes, but does contain VPIΦ genes (Table 1). Our CVD110 preparation contained many phage particles, some of which formed a 'braided' network of filaments, presumably representing VPIΦ (Fig. 5b). Immunoelectron microscopy of phage preparations from El Tor strain N16961, like 395, revealed numerous gold particles bound to filamentous phage (Fig. 5c).

As VPI is a bacteriophage and the structure of type-IV pili resembles that of a filamentous bacteriophage¹⁶, we investigated whether the TCP pilin subunit, TcpA, could be a VPIΦ coat protein. TCP2 is a derivative of strain 395 with a large internal deletion in the *tcpA* gene that prevents it from producing TCP¹⁷. No VPIΦ genes were detected in phage preparations from TCP2, indicating that TcpA is required for VPIΦ production; in contrast, CTXΦ genes were evident (Table 1 and Fig. 2). In immunoprecipitation experiments on these phage preparations, rabbit anti-TcpA-peptide antibodies and mouse anti-rabbit agarose beads bound VPIΦ, allowing selective removal of the complex. PCR analysis on N16961 and 395 immunoprecipitates revealed VPIΦ-encoded genes (Fig. 6). We

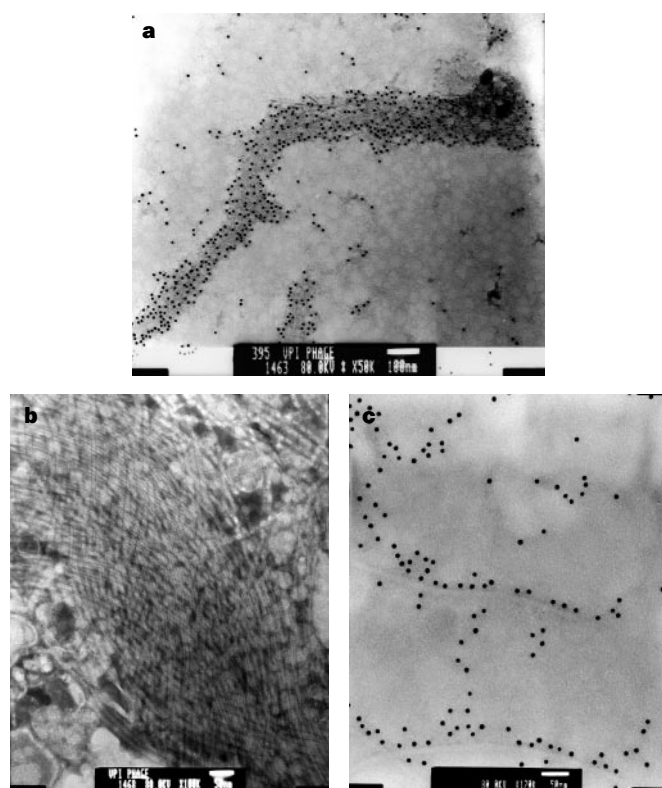


Figure 5 Electron micrographs of VPIΦ. **a**, Phage preparations from 395 incubated with rabbit anti-TcpA antibody and 10-nm colloidal gold-conjugated goat anti-rabbit IgG (original magnification, $\times 50,000$; scale bar, 100 nm); **b**, phage preparations from CVD110 (magnification, $\times 100,000$; scale bar, 50 nm); **c**, Phage preparations from N16961 incubated with rabbit anti-TcpA antibody and 10-nm colloidal gold-conjugated goat anti-rabbit IgG (magnification $\times 120,000$; scale bar, 50 nm).

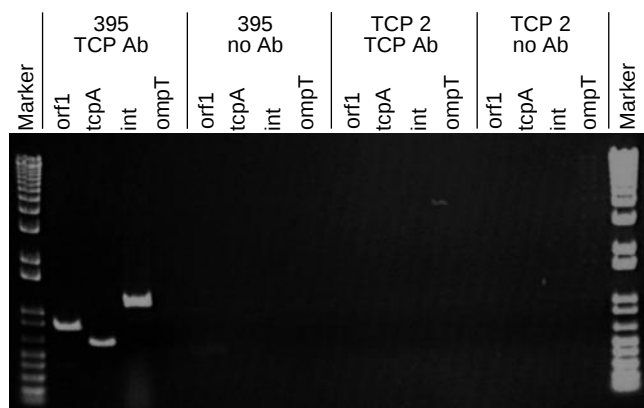


Figure 6 PCR products of immunoprecipitated phage preparations from strains 395 and TCP2, following incubation with and without anti-TCP antibodies (Ab), demonstrate specificity of anti-TCP antibody for VPIΦ. Genes amplified by PCR are indicated above each well.

Table 2 Detection of genes in phage immunoprecipitation reactions

Strain	Sample	Detection of genes							
		<i>orf1</i>	<i>tcpA</i>	<i>toxT</i>	<i>int</i>	<i>ctxB</i>	flank VPI	<i>ompT</i>	<i>rfaD</i>
395	Anti-TCP	+	+	+	+	—	—	—	—
	Bead only	—	—	—	—	—	—	—	—
TCP2	Anti-TCP	—	—	—	—	—	—	—	—
	Bead only	—	—	—	—	—	—	—	—

PCR was used on phage preparations from 395 (wild type) and TCP2 (*tcpA* mutant) immunoprecipitated in the presence or absence of anti-TCP antibody.

demonstrated antibody specificity for TcpA and VPI Φ by the lack of PCR products in similar experiments done without anti-TcpA antibodies (Table 2). PCR on phage preparations from an independent *tcpA* mutant, RT4032, which contains an in-frame (non-polar) deletion in *tcpA*, also failed to generate VPI genes; however, the *tcpA* mutants TCP2 and RT4032 could be complemented by supplying *tcpA* on a plasmid (pRT198) because PCR detected VPI genes in phage preparations of both transformants (Fig. 7). These results together support the idea that TcpA is an important coat protein of VPI Φ .

Our findings help explain how non-pathogenic bacteria can become pathogens. Many bacterial pathogens contain clusters of genes that encode virulence factors responsible for inducing disease. We have shown that the large cluster of genes essential for the epidemic properties of *V. cholerae* originates from viral (bacteriophage) DNA that has become incorporated into the bacterial chromosome, and that transfer of the unusually large filamentous bacteriophage VPI Φ confers new virulence on the recipient. Type IV pili like TCP are expressed by a variety of human and animal pathogens, including enteropathogenic *E. coli*, *Neisseria gonorrhoeae*, *P. aeruginosa* and *D. nodosus*^{18–21}, and may also have a bacteriophage origin and transferable genes that endow the bacterium with virulence factors. It is not understood how TCP serves as both a bacteriophage and colonization factor, particularly as it has not been shown to bind directly to intestinal tissue or to cultured epithelial cells. The type-IV pilus of enteropathogenic *E. coli*, BFP, which shares significant homology with TCP, was recently reported to function in colonization by mediating bacteria-to-bacteria adherence, thereby increasing the bacterial mass that colonizes the

intestine²². TCP likewise mediates interbacterial adherence (auto-agglutination is a standard assay for this structure), so released VPI Φ /TCP pili might help colonization by serving as a bridge between phage/pili bound to different bacteria. TCP is also the receptor for CTX Φ (ref. 4), creating a situation in which one phage serves as the receptor for a second phage in a sequential infection process that results in bacterial virulence. Our results highlight the potential contribution of virulence-conferring phage ('pathophage') in the emergence of pathogens, now and in the future. □

Methods

Bacterial strains and plasmids. Strain 395 is a representative of the sixth pandemic clone (classical biotype) of *V. cholerae*; strain N16961 is an isolate from the current seventh ('El Tor') pandemic which began in 1961 in Indonesia. CVD110 is a derivative of a seventh pandemic strain (E7946) which is deleted in its *ctxA*, *zot*, *ace* and *orfU* genes¹⁵. The environmental isolates DK236 and DK237 are non-toxigenic VPI-negative serogroup O10 and O1 strains, respectively, and are Nal-resistant owing to selection of a spontaneous resistant mutant. To construct DK238, the *xtn-aldA* region of the chromosomally integrated N16961 VPI was amplified by PCR with primers KAR166 (located in *orf1*) and KAR167 (located in *aldA*). This 1.8-kb fragment was ligated into pGEM-T, creating pDK40. This plasmid was digested with *Bam*HI and the *aphA*-3 gene (encoding Km/Neo resistance) was inserted, creating pDK42, which was digested with *Sph*I/*Sac*I; the *xtn-aldA::aphA*-3 fragment was ligated into the suicide vector pCVD442, creating pDK43. Plasmid pDK43 was used in an allelic exchange procedure to introduce the *aphA*-3-containing fragment into the homologous region of the N16961 chromosome, creating strain DK238. Strain TCP2 is derived from 395, has amino-acid residues 119–154 deleted from its *tcpA* gene and does not produce the TCP structure¹⁷. RT4032 (from R. Taylor) is an in-frame *tcpA* deletion mutant of 395 in which the codons encoding amino acids +1 of mature TcpA through the TAA stop codon and one additional T nucleotide are removed. Plasmid pRT198 is a pBR322-based plasmid with a 2-kb *Hind*III fragment containing *tcpA* of 395 cloned into the *Hind*III site of pBR322.

PCR and sequencing. PCR was performed as described²³ under the following conditions: denature at 96 °C for 3 min; annealing, 48 °C, 30 s; extension, 72 °C, 2 min for 1 cycle, then denature 96 °C, 30 s; annealing, 48 °C, 30 s; extension, 72 °C, 2 min for 30 cycles. Primers used to determine the presence and sequence of *V. cholerae* genes were: *orf1*, KAR96, 5'-TGCTACTTACCAATGGCAC-3' and KAR97, 5'-GAGCCAGGCTTATTTGGGCG-3'; *tcpA*, KAR24, 5'-AAAA CCGGTCAAGAGGG-3' and KAR25, 5'-CAAAAGCTACTGTGAATGG-3' for seventh pandemic strains and KAR82, 5'-CAAATGCAACGCCGAATGG-3' for sixth pandemic strains; *toxT*, KAR90, 5'-ATACTTTACGTGGATGGC-3' and KAR91, 5'-AAAATCAGTGATACAATCG-3'; *int*, KAR22, 5'-GATAAAGAGAT CAAAGCC-3' and KAR23, 5'-ATCTGCTTCCATGTGGG-3'. Additional primers included KAR94, 5'-TATGATACTGAAACACCTC-3' and KAR95, 5'-GATGCTAACAGCAGAGCATA-3' (outside left VPI junction); KAR85, 5'-CGCCTGCCAACCAGACACGC-3', and KAR86, 5'-GCAGCAAGCCTCCACT CCG-3' (outside the right VPI junction); K898, 5'-GAATTCTGTC GGGTTGTAATCCTG-3' and K643, 5'-GCCATACTCAGCATATACAC-3' (*ompT*); K371, 5'-CGGGATCCGAGCTCATTACCTACACTAGTG-3' and K369, 5'-CGGGATCCGACAGGCTATAATGCGTGCAAC-3' (*rfaD*); KAR161, 5'-AAAATTCCTTGACGAATACC-3' and KAR162, 5'-TTGCTTCTCATCAT CGAACC-3' (*ctxB*); KAR166, 5'-GACAGGATTACTGAGATATCTG-3' and KAR167, 5'-AACCAGGTGAGGTTGTACC-3' (*xtn-aldA* region). Primers were synthesized using an Applied Biosystems DNA synthesizer and sequenced

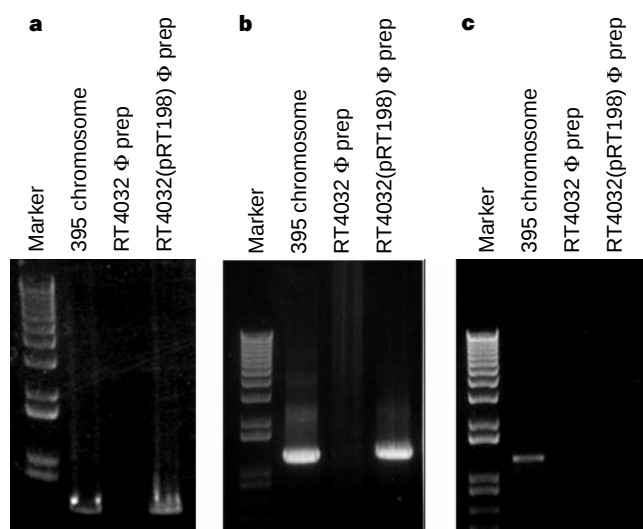


Figure 7 PCR analysis of VPI genes from a *tcpA* mutant and mutant complemented with *tcpA*. **a**, PCR done with *tcpA* primers KAR24 and KAR82; **b**, PCR done with *orf1* primers; **c**, PCR done with *rfaD* primers. Note the absence of a *tcpA* and *orf1* PCR product in phage preparations from RT4032 (owing to its inability to make VPI Φ) and the presence of products when RT4032 is complemented with pRT198 supplying *tcpA*.

with a *Taq* Dye-Terminator kit (Perkin-Elmer) and an automated 373A DNA sequencer (Applied Biosystems).

Isolation of phage and replicative form. Isolation of phage from *V. cholerae* was done essentially as described²⁴. In brief, 1-litre Luria broth cultures were grown overnight at either 30°C (395) or 37°C (N16961). Cultures were centrifuged twice at 10,000 g and the supernatant was passed through a 0.45-µm low-protein-binding filter. DNase I and RNase I (Boehringer Mannheim) were added to the filtrate at a final concentration of 1 µg ml⁻¹ and incubated at room temperature for 3 h. NaCl and PEG 8000 were added to a final concentration of 1 M and 10% w/v, respectively, and the mixture was left to precipitate overnight at 4°C. The supernatant was centrifuged at 11,000 g for 20 min and the pellet resuspended in 4 ml of SM buffer. PEG was removed with an equal volume of chloroform. This supernatant was layered onto a CsCl₂ step gradient consisting of 2 ml each of CsCl₂ in SM buffer ($d = 1.7, 1.5$ and 1.45). After centrifugation at 25,000 r.p.m. for 2 h in an SW41 rotor (Beckman), the lower phage band ($1.45 \leq d \leq 1.5$) was extracted and dialysed against two changes of TM buffer. The phages were further concentrated by the addition of PEG 8000 (10% w/v) and placed on ice for 2 h. The phage preparation was centrifuged at 14,000 g for 20 min and the resulting pellet (containing phage particles) was resuspended in 100 µl of SM buffer. PEG was removed with an equal volume of chloroform. 5 µl of the phage preparation was used for PCR.

The replicative form was isolated from 1 litre of Luria broth culture as described²⁴.

Although filamentous phage do not lyse the cell or have a 'burst size', we calculated the approximate number of VPIΦ and CTXΦ released per cell and the RF copy number. The number of phage released was calculated by determining the amount of phage DNA (from its absorbance) in 1 litre of overnight culture of DK238 and CVD110 containing ~10¹² cells. As strain DK238 is positive for VPIΦ and CTXΦ and CVD110 is positive for only VPIΦ, the difference in amount (in µg) between the two strains should reflect the number of CTXΦ genomes (7 kb) and the balance the number of VPIΦ genomes (40 kb) released per cell. We estimate that 280 and 200 µg l⁻¹ of ssDNA is present in a phage preparation from 1-litre cultures of DK238 and CVD110, respectively. If 1 µg of 1-kb ssDNA contains 1.8×10^{12} molecules, then 1 µg of 7-kb ssDNA contains 2.6×10^{11} CTXΦ molecules and 80 µg contains 2×10^{13} molecules, so 2×10^{13} molecules/10¹² cells indicates that an average of 20 CTXΦ are made per cell during overnight culture. Likewise, for VPIΦ we calculate that an average of 9 VPIΦ are produced per cell. Our calculations all assume that no other phage (or RF) is produced and that DNA extraction is 100% efficient, which is unlikely, so values could be underestimates.

We estimated that 57 and 39 µg l⁻¹ of dsDNA are present in RF preparations from 1-litre cultures of DK238 and CVD110, respectively, which yields an average CTXΦ RF copy number of 2 per cell after overnight culture; the average VPIΦ RF copy number is similarly estimated as 1 per cell.

Immunoprecipitation. DNase- and RNase-treated phage preparations were incubated with rabbit anti-TcpA peptide antibody (1:10,000) at 37°C and with vigorous shaking for 1 h. Mouse anti-rabbit IgG (whole molecule) agarose beads (Sigma) were added and the reaction was incubated at 4°C overnight with gentle rotation. After several low-speed centrifugations and washings in deionized water, the pellet was resuspended in deionized water.

Electron microscopy. Phage preparations of CVD110 and 395 were placed on a carbon-formvar-coated 300-mesh copper grid (Electron Sciences) for 2 min then negatively stained with 1.5% phosphotungstic acid for 1 min and analysed by electron microscopy. In addition, equal volumes of 395 and N16961 phage preparations were separately incubated with rabbit anti-peptide TcpA antibody (1:10,000) at 37°C for 20 min, after which this suspension was placed on a grid for 5 min, and 10 µl of 10-nm colloidal gold-conjugated goat anti-rabbit IgG (ICN Biomedicals) was added before negative staining.

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Structure of Cdc42 in complex with the GTPase-binding domain of the 'Wiskott-Aldrich syndrome' protein

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The Rho-family GTP-hydrolysing proteins (GTPases), Cdc42, Rac and Rho, act as molecular switches in signalling pathways that regulate cytoskeletal architecture, gene expression and progression of the cell cycle¹. Cdc42 and Rac transmit many signals through GTP-dependent binding to effector proteins containing a Cdc42/Rac-interactive-binding (CRIB) motif². One such effector, the Wiskott-Aldrich syndrome protein (WASP), is postulated to link activation of Cdc42 directly to the rearrangement of actin³.

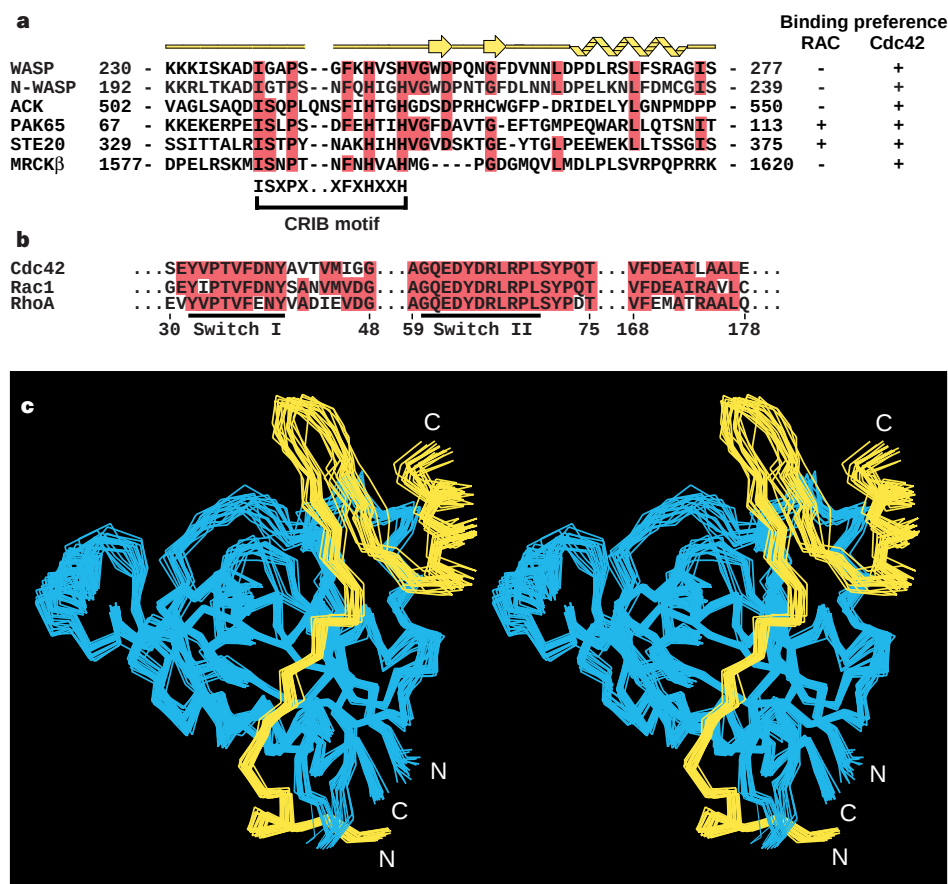


Figure 1 Sequence alignment and structure overlay of the Cdc42-WASP complex. **a**, Sequence alignment of WASP (residues 230–277) with similar regions of selected CRIB-containing proteins. Qualitative GTPase binding preferences are listed at right. Residues appearing in four or more sequences are boxed in red. Consensus CRIB motif is indicated below the alignment. Consensus WASP secondary structure²⁹ observed in the Cdc42 complex is shown above. **b**, Partial alignment of the human sequences of Cdc42, Rac and

Rho. Residue numbers for Cdc42/Rac are displayed below the alignment; values for Rho are 2 greater. Switch regions I and II are underlined. **c**, Stereoview of the best-fit superposition of the backbone (N, Cα, C) atoms of the 20 final NMR structures of the Cdc42 (blue)/WASP (yellow) complex. Residues 278–288 of WASP show no long-range NOEs, appear disordered in solution, and have been removed for clarity. Nucleotide is not shown.

Human mutations in WASP cause severe defects in haematopoietic cell function, leading to clinical symptoms of thrombocytopenia, immunodeficiency and eczema. Here we report the solution structure of a complex between activated Cdc42 and a minimal GTPase-binding domain (GBD) from WASP. An extended amino-terminal GBD peptide that includes the CRIB motif contacts the switch I, β2 and α5 regions of Cdc42. A carboxy-terminal β-hairpin and α-helix pack against switch II. The Phe-X-His-X₂-His portion of the CRIB motif and the α-helix appear to mediate sensitivity to the nucleotide switch through contacts to residues 36–40 of Cdc42. Discrimination between the Rho-family members is likely to be governed by GBD contacts to the switch I and α5 regions of the GTPases. Structural and biochemical data suggest that GBD-sequence divergence outside the CRIB motif may reflect additional regulatory interactions with functional domains that are specific to individual effectors.

To identify a suitable system for structural analysis, we assayed the binding of a series of WASP fragments to Cdc42 in complex with the non-hydrolysable GTP analogue GMPPCP and Mg²⁺, both biochemically and by NMR spectroscopy. All fragments contained the WASP CRIB motif, amino acids 238–251, defined as the minimum conserved sequence in several Cdc42/Rac-binding proteins² (Fig. 1a). As found previously⁴, only fragments containing at least residues 230–288 of WASP maintained high affinity for the GTPase (K_D, 20–30 nM). NMR analyses indicated that all unbound peptides were largely disordered in solution. However, in the presence of

Cdc42-GMPPCP, they adopted discrete structure, with a minimal binding domain indicated by a conserved group of 44 well dispersed cross-peaks in ¹H/¹⁵N heteronuclear single-quantum coherence spectra. We carried out structural studies on a fragment of WASP (amino-acids 230–288) bound to Cdc42 (residues 1–179) in complex with GMPPCP and Mg²⁺. Table 1 shows statistics describing the final set of 20 converged structures. A best-fit backbone superposition of the conformers is shown in Fig. 1c.

Residues in the CRIB motif bind Cdc42 in an extended conformation, contacting the five C-terminal residues of switch I, and the entire adjacent β2 strand (residues 41–47; Fig. 2a). This interaction effectively appends the central β-sheet of the GTPase with an additional, irregular strand containing a series of conserved β-bulges at WASP residues 240–241, 243–244 and 247–248. Binding through strand-like contacts is also found in the two reported Ras-family GTPase-effector complexes, Rap1A–Raf (ref. 5) and Ras–RalGDS (ref. 6) (Fig. 2b), indicating that this may be a common feature of target recognition by small GTPases. However, in these cases binding involves an independently folded domain of the effector, and the contacts cover a larger region of switch I without extending across β2. N-terminal to the CRIB region, WASP residues 231–237 form a compact loop that packs against the β2–β3 turn and adjacent C-terminal α5 helix of Cdc42. The C-terminal portion of the GBD is folded into a two-stranded antiparallel β-hairpin (residues 252–253 and 257–258) and a short α-helix (residues 265–274). Both of these elements are amphipathic and contact a

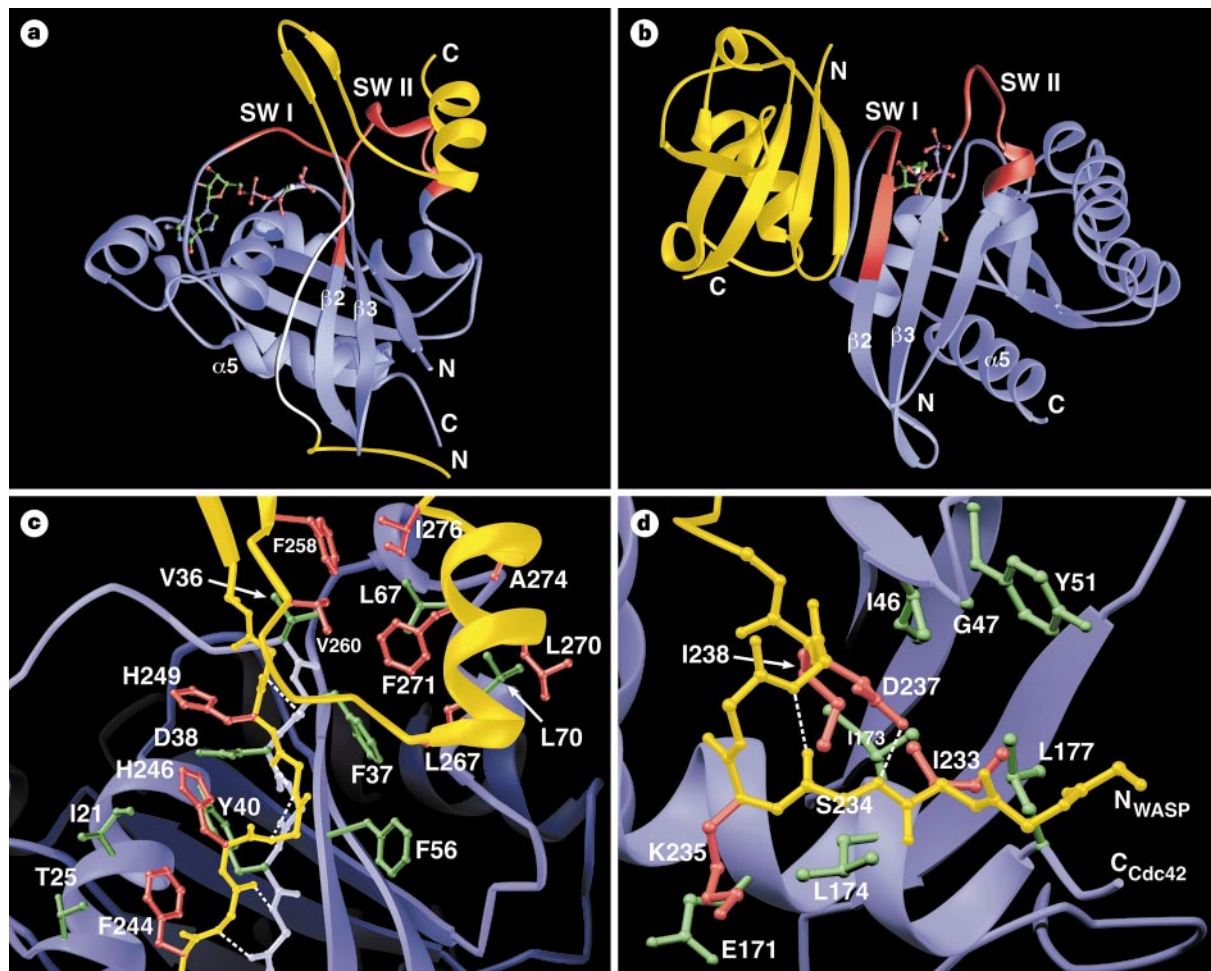


Figure 2 GTPase-effector interactions. **a**, Ribbons³⁰ depiction of a representative conformer from the final ensemble of structures of the Cdc42 (blue)/WASP (yellow) complex. Switch I (residues 32–40) and switch II (residues 60–70) of Cdc42 are red. CRIB motif of WASP is white. **b**, Rap1A/Raf complex⁵ coloured as in **a**. Nucleotide and Mg^{2+} in **a** and **b** are displayed as ball and stick models. **c**, Contacts between WASP (yellow with red side chains) and the switch I, switch II

and $\beta 3$ regions of Cdc42 (blue with green side chains). Intermolecular main-chain hydrogen bonds observed in most members of the NMR ensemble are indicated by dashed lines. Nucleotide not shown. **d**, Interaction of the WASP GBD N terminus and the Cdc42 $\beta 2/\beta 3$ hairpin and $\alpha 5$ helix, coloured as in **c**. Intramolecular hydrogen bonds between GBD residues 234 and 237 are indicated by dashed lines.

hydrophobic patch on the surface of Cdc42 composed of the C-terminal portions of switch I, switch II and strand $\beta 3$ (Fig. 2a, c). Figure 3 shows intermolecular nuclear Overhauser effects (NOEs) that define packing of the switch-II residues Leu 67 and Leu 70 against aliphatic and aromatic residues of the WASP β -hairpin and helix (Phe 258, Leu 267, Leu 270, Phe 271, Ala 274 and Ile 276). The extensive Cdc42–WASP interface results in burial of over twice as much total surface area as in the Rap1A–Raf (ref. 5) complex ($2,930 \pm 60 \text{ \AA}^2$ versus $1,270 \text{ \AA}^2$).

The WASP GBD has about 500-fold greater affinity for Cdc42–GTP than for Cdc42–GDP (ref. 4). Although high-resolution structures of the two Cdc42 states are not available, chemical shift comparisons⁷, as well as analogy to the Ras system, indicate that conformational differences will be localized primarily to switch I and switch II. Thus, contacts to these regions will mediate the sensitivity of WASP binding to the nucleotide switch. Cdc42 residues Phe 37, Asn 39 and Ala 41, near the C terminus of switch I, form main-chain hydrogen bonds to WASP residues Val 250, Val 247/Ser 248 and Lys 245, respectively (Fig. 2c). These interactions position the Asp 38 side chain of Cdc42 near WASP residues His 246 and His 249. There is a hydrogen bond from the Asp 38 carboxylate to either the His 246 or His 249 imidazole ring in nearly all members of the NMR ensemble. Disruption of these contacts

through mutation of either the GTPase (for example, Asp 38 to glutamate in Cdc42; ref. 8) or effector (for example, His 208 to aspartate in N-WASP; ref. 9) significantly (>50-fold for Asp 38 to glutamate) decreases the affinity of Cdc42 and Rac for CRIB proteins *in vitro* and *in vivo*, explaining the high conservation of the two histidine residues throughout the CRIB effector family. Sheet-like intermolecular hydrogen bonds also position the side chain of conserved WASP residue Phe 244 in a shallow hydrophobic pocket between $\alpha 1$ and $\beta 2$ of Cdc42 formed by Ile 21, Thr 25 and Tyr 40. The importance of these interactions was shown by mutagenesis of Tyr 40 to cysteine or lysine in Cdc42 or Rac, which substantially decreases their affinity for CRIB-containing targets¹⁰.

The side chains of switch I residues Val 36 and Phe 37 form one side of an exposed hydrophobic surface of Cdc42, with Phe 56 in $\beta 3$, and Leu 67 and Leu 70 in switch II. This site interacts with the hydrophobic faces of the GBD C-terminal β -hairpin and helix (Fig. 2c) with numerous intermolecular NOEs from Phe 37 and Phe 56 to WASP residues Val 260, Leu 263 and Leu 267 (see also Fig. 3). These contacts are significant to recognition, as elimination of the C-terminal GBD helix decreases affinity for Cdc42 approximately six-fold⁴. Preferential binding of WASP to Cdc42–GTP thus appears to derive in part from polar and hydrophobic contacts involving highly conserved residues in the CRIB motif. Hydrophobic

contacts outside the CRIB motif also appear to be important in response to the nucleotide switch.

CRIB-family proteins bind activated Cdc42 and Rac, but not Rho². Within the family there are variable levels of additional selectivity: proteins such as WASP, ACK and MRC kinase preferentially bind Cdc42 over Rac^{3,11,12}, whereas others such as PAK discriminate little between the two GTPases². The Cdc42–WASP structure allows us to understand some aspects of these specificities. The three Rho-family members are identical in the switch I and switch II portions of the WASP-binding site, except at position 38, which is aspartate in Cdc42 and Rac but glutamate in Rho (Fig. 1b). Surprisingly, mutation of Asp 38 in Rac to glutamate⁸ decreases the affinity for the PAK GBD by a factor of 50, probably by disrupting contacts to the conserved HXXH motif in the CRIB region, as already described. Thus, this single conservative difference appears to be important for mediating CRIB specificity against Rho.

Biochemical evidence and sequence comparisons indicate that there may be additional specificity determinants in the C-terminal $\alpha 5$ helix of the GTPase¹³. Hydrophobic interactions anchor WASP residues 232–238 to the Cdc42 $\beta 2/\beta 3$ hairpin and $\alpha 5$ helix (Fig. 2d). WASP residue Ile 233, which is a hydrophobic amino acid in most CRIB proteins, packs into a hydrophobic pocket formed by Cdc42 residues Ile 46, Tyr 51, Leu 177 and Leu 174, and the highly conserved Ile 238 packs against Cdc42 residues Ile 46 and Ile 173. A β -turn in the GBD, stabilized by backbone and side-chain hydrogen bonds between Asp 237 and Ser 234, then packs the hydrophobic portion of the Lys 235 sidechain of WASP against the Cdc42 Leu 174 sidechain. This arrangement positions the Lys 235 amine close to the carboxylate of Cdc42 Glu 171, facilitating hydrogen-bond formation. In Rac and Rho, Leu 174 is replaced by arginine, and Gly 47 in the $\beta 2/\beta 3$ hairpin is replaced by aspartate. Hydrogen bonding between these side chains, which is seen in activated Rac¹⁴ and Rho¹⁵, closes off the pocket contacted by Ile 233 of WASP, and would disrupt the hydrophobic contacts to Lys 235. Thus, these differences may enhance the ability of WASP to distinguish Cdc42 from Rac and Rho. PAK, which has a similar affinity for Cdc42 and Rac,

contains a lysine at the position corresponding to Ile 233 of WASP (Fig. 1a), suggesting that it may interact differently with the GTPase in these regions. Thus, the extended nature of the WASP–GTPase interface may enable binding energy and specificity elements to be distributed in regions of Cdc42 that are modulated by nucleotide exchange and in more static portions of the molecule. In contrast, in the Ras family, nucleotide exchange and selectivity are intimately coupled, with a single residue in switch I at position 31 of Ras and Rap1A largely governing discrimination between Raf and RalGDS (ref. 16).

The Cdc42–ACK structure¹⁷ shows similar interactions throughout the CRIB motif to those observed for Cdc42–WASP, with both effectors extending the GTPase β -sheet by an additional strand. However, interactions in regions outside the CRIB motif, particularly in the C termini of the two GBDs, are quite different. Such variability in extra-CRIB interactions may be a common theme in structures throughout the effector family. Sequence conservation outside the CRIB motif is very low (Fig. 1a), and biochemical and structural data indicate that, like ACK, PAK contacts switch I more extensively than we observe for WASP. Thus, whereas PAK binding strongly inhibits nucleotide dissociation from Cdc42 (ref. 8), WASP has only a fourfold effect⁴. In addition, covalent modification of the ribose ring of bound nucleotide (proximal to Glu 31/Tyr 32) decreases the affinity of PAK for Cdc42 approximately 30-fold⁸, but has no effect on WASP binding⁴. Finally, an intermolecular NOE has been reported from the N-terminal region of switch I in Cdc42 to bound PAK¹⁸ that is incompatible with the binding mode in the WASP complex. The functional explanation for these differences may lie in the specific mechanisms of signalling through WASP and PAK. In N-WASP, the GBD binds to and inhibits the actin depolymerization activity of an acidic C-terminal domain, an interaction postulated to involve the highly basic region immediately N-terminal to the CRIB motif¹. This inhibition is relieved through competitive binding of Cdc42 to the GBD. Similarly, in PAK, C-terminal regions of the GBD appear to regulate the serine/threonine kinase domain of the protein negatively, as mutation of

Table 1 Structural statistics

Restrains for structure calculation	
Total restraints	4,180
Total NOE restraints	3,738
Unambiguous intra WASP/Cdc42	784/2,146
Unambiguous inter WASP–Cdc42	322
Ambiguous	486
Dihedrals (ϕ , ψ , χ_1) WASP/Cdc42	(32,16,9)/(97,97,59)
Statistics for structure calculation	
R.m.s.d. from experimental restraints*	
Distances (Å)	0.019 $\pm$ 0.001
Angles (deg)	0.32 $\pm$ 0.10
Deviations from idealized geometry	
Bonds (Å)	0.002 $\pm$ 0.001
Angles (deg)	0.40 $\pm$ 0.01
Final energies (kcal mol ⁻¹)	
E_{total} , E_{NOE} , E_{cdih}	420 $\pm$ 19, 67 $\pm$ 6, 2.3 $\pm$ 1.5
Coordinate precision† (Cdc42 1–179/WASP 231–277)	
R.m.s.d. of backbone atoms (N, C α , C')	0.79 $\pm$ 0.11 Å
R.m.s.d. of all heavy atoms	1.15 $\pm$ 0.11 Å
Procheck analysis (Cdc42 1–179/WASP 231–277)	
Most favoured	62.6/77.5
Additional allowed	35.5/19.5
Generously allowed	1.3/2.4
Disallowed region	0.6/0.5

Force constants used in the simulated annealing target function are: 5 kcal mol⁻¹ Å⁻⁴ for the quartic van der Waals repulsion term (with atomic radii set to 0.78 times their value defined in the paramllhdg-pro parameter set), 50 kcal mol⁻¹ Å⁻² for distance restraints, 200 kcal mol⁻¹ rad⁻² for torsion angle restraints.

*None of the final structures has distance restraint violations greater than 0.3 Å or dihedral violations greater than 5 deg.

†Defined as average root-mean-squared difference for the stated residues between the final 20 structures and the average coordinates.

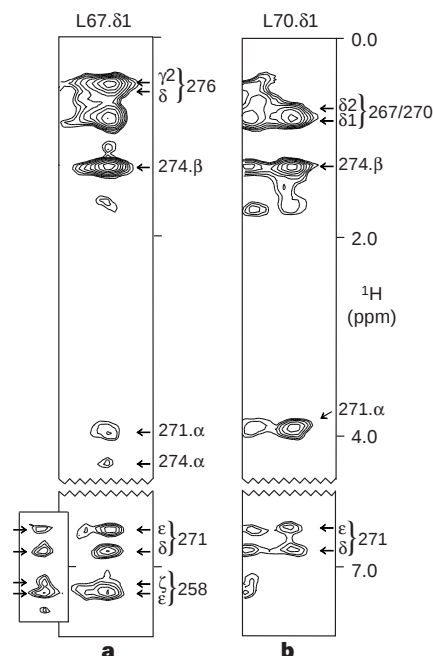


Figure 3 Selected regions of a ¹³C-filtered NOESY spectrum recorded in D₂O. Intermolecular NOEs are shown to the C^{δ1}H₃ methyl groups of **a**, Leu 67, and **b**, Leu 70 of Cdc42. Unambiguous WASP assignments are indicated on the side. Inset in **a** displays NOEs from Leu 67 C^{δ1}H₃ to aromatic protons of WASP observed in an analogous spectrum recorded on a complex between ¹³C-labelled, methyl-protonated but otherwise deuterated, Cdc42 and unlabelled WASP.

Leu 107, or proteolytic removal of N-terminal regulatory sequences, including the GBD, lead to constitutive activation^{19,20}. Thus, sequence divergence in the extra-CRIB regions, and the resulting variation in GTPase contacts, may reflect additional regulatory interactions with other enzymatic/functional domains in the effectors. Structural overlap between the intermolecular (GTPase) and intramolecular (enzymatic domain) binding sites of the GBD, and its thermodynamic consequences, could be subtle, and themselves subject to additional regulation. In both WASP and PAK, the GTPase-binding and enzymatic domains are separated by proline-rich sites that bind SH3 domains^{21,22}. Ligation of these sites could modulate the thermodynamic balance in favour of activation or inhibition. Additional structures of CRIB proteins, both free and in complex with GTPases, will be needed to understand these thermodynamic and structural aspects of recognition and signalling through the Rho-family GTPases. The structure of a Cdc42–WASP–GBD complex represents a first step in this direction. □

Methods

Sample preparation. WASP and Cdc42 were overexpressed from vector pET11a in *Escherichia coli* strain BL21(DE3), and purified from bacterial lysate chromatographically. Uniform ¹⁵N and ¹³C labelling was achieved by growing bacteria in minimal medium supplemented with ¹⁵NH₄Cl and/or ¹³C₆-glucose as the sole nitrogen and carbon sources. Deuteration was achieved by growth in 50–99.9% D₂O with protonated (50–70% ¹H samples) or deuterated (99.9% ²H samples) ¹³C₆-glucose. Selective methyl protonation of leucine, valine and isoleucine (δ1) in a deuterated background was obtained as described²³.

NMR spectroscopy. Spectra were recorded at 25 °C on Varian Inova 600 or 800 MHz spectrometers. Sequential assignments were obtained through the following ²H-decoupled, triple-resonance spectra recorded on complexes with one unlabelled component and one ²H(90%)/¹⁵N/¹³C-labelled component: CT–HNCa, CT–HN(CO)Ca, HN(Ca)Cb, HN(COCa)Cb, HNCO, HN(Ca)CO (ref. 24). Side-chain resonances were assigned using HCC–TOCSY, CCC–TOCSY–NNH and HCC–TOCSY–NNH experiments recorded on protonated and 50–70% random fractionally deuterated samples. Leucine, valine and isoleucine (δ1) methyl assignments for Cdc42 were obtained from a methyl-protonated, otherwise fully deuterated sample bound to unlabelled WASP using optimized HCC- and CCC–TOCSY–NNH experiments²⁵. Aromatic protons were assigned through 2D ¹H–NOESY and ¹H–TOCSY spectra recorded in D₂O on complexes with one protonated component and one deuterated component. NOEs were obtained from three-dimensional ¹⁵N-edited, ¹⁵N/¹³C-edited, ¹⁵N-edited–¹⁵N/¹³C-filtered and ¹³C-edited–¹⁵N/¹³C-filtered NOESY spectra (τ_{mix} of 40, 75 ms) on complexes having only a single ¹⁵N/¹³C-labelled component. Intermolecular NOEs identified in a complex of ¹⁵N/¹³C–Cdc42 and ¹H–WASP were confirmed using a sample with ¹H–Cdc42 and ¹⁵N/¹³C–WASP. Intermolecular NOEs from Cdc42 methyl groups to WASP aromatic protons were confirmed through a ¹³C-edited–¹³C-filtered NOESY spectrum recorded on a complex of ²H/¹³C/methyl protonated Cdc42 and unlabelled WASP.

Structure determination. For initial calculations, we used a torsion angle dynamics simulated annealing protocol implemented in CNS²⁶ to dock the GBD onto coordinates of Cdc42–GMPPNP derived from the crystal structure of the GTPase in complex with GMPPNP–Mg²⁺ and rhoGAP (unpublished coordinates provided by N. Nassar and R. Cerione) through 382 intra-WASP and 170 intermolecular NOEs. These Cdc42 coordinates are similar to those of Rac–GMPPNP–Mg²⁺ and Rho–GTPγS–Mg²⁺ (backbone r.m.s.d. of 0.88 Å and 1.01 Å, respectively)^{14,15}. Structures from these calculations formed the basis for automated, iterative assignment of intra-Cdc42 and remaining intra-WASP NOEs using ARIA²⁷. In addition to NOE data, calculations used 128 restraints for 64 intra-Cdc42 hydrogen bonds found in regular secondary structure elements in both the Cdc42–GMPPNP–Mg²⁺–rhoGAP and Rac–GMPPNP–Mg²⁺ structures¹⁴. Internal GTPase side chains in 55 residues, whose amide chemical shifts were not perturbed by WASP binding, were restrained to within ±20–60° of their rotamers observed in the Cdc42–GMPPNP–rhoGAP crystal structure. Nucleotide and Mg²⁺ coordinates were fixed in the calculations, and restrained to Cdc42 by hydrogen bonds conserved in Ras-like GTPase structures. The χ1 torsions of nine WASP and four Cdc42 side

chains at the molecular interface were restrained to ±20–40° based on patterns of local NOEs. A total of 16 WASP ϕ angles were restrained to ±40° based on qualitative analysis of an HNHa–J experiment. We performed six rounds of calculations with ambiguous cut-off parameter, *p* (ref. 27), decreasing from 0.96 to 0.7. This was repeated several times, as intermolecular NOEs were added manually. Late in the refinement, we included 97/16 (Cdc42/WASP) ϕ and 97/26 (Cdc42/WASP) ψ restraints, based on analysis of chemical shifts in the program TALOS²⁸. Dihedrals were restrained to the maximum of 30° or twice the standard deviation observed in the TALOS database matches. We assessed structure quality with PROCHECK–NMR²⁹.

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Structure of the small G protein Cdc42 bound to the GTPase-binding domain of ACK

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The proteins Cdc42 and Rac are members of the Rho family of small GTPases (G proteins), which control signal-transduction pathways that lead to rearrangements of the cell cytoskeleton, cell differentiation and cell proliferation. They do so by binding to downstream effector proteins¹. Some of these, known as CRIB (for Cdc42/Rac interactive-binding) proteins², bind to both Cdc42 and Rac, such as the PAK1–3 serine/threonine kinases³, whereas others are specific for Cdc42, such as the ACK tyrosine kinases^{4,5} and the Wiscott–Aldrich-syndrome proteins (WASPs)^{6,7}. The effector loop of Cdc42 and Rac (comprising residues 30–40, also called switch I), is one of two regions which change conformation on exchange of GDP for GTP. This region is almost identical in Cdc42 and Rac, indicating that it does not determine the specificity of these G proteins. Here we report the solution structure of the complex of Cdc42 with the GTPase-binding domain of ACK^{4,5}. Both proteins undergo significant conformational changes on binding, to form a new type of G-protein/effector complex. The interaction extends the β -sheet in Cdc42 by binding an extended strand from ACK, as seen in Ras/effector interactions^{8,9}, but it also involves other regions of the G protein that are important for determining the specificity of effector binding.

Residues 499–551 of ACK bind Cdc42 with a similar affinity to that of the full-length ACK protein⁴. We expressed residues 504–545 of ACK (f-ACK; Fig. 1a), which also binds Cdc42 with high affinity (Table 1), and a truncated form of Cdc42. The structures of free Cdc42 in complex with the GTP analogue GMPPNP and its f-ACK complex were then determined using NMR spectroscopy (Fig. 2). The spectra of free Cdc42–GMPPNP were of high quality, but there were no resonances for residues 32 and 35–40 (in switch I) and residues 56–60, 65 and 66 (in switch II), the two regions that change conformation upon GDP/GTP exchange (Figs 1b, 3a). These regions are therefore flexible, exchanging conformations on a time-scale that leads to signal broadening. Resonances from switch I and most of switch II were present in spectra of the complex, showing that their conformations become fixed when f-ACK binds. The positions of resonances from residues around switch I and II, the three-stranded β -sheet and helix α 5 also shifted. In contrast to the

free GTPase-binding domain (GBD) of WASP, which contains an α -helix at its carboxy terminus¹⁰, the NMR spectra of free f-ACK indicate that it is largely unstructured in the absence of Cdc42.

The structure of the complex was calculated using 3,097 nuclear Overhauser effect (NOE) distance restraints (2,566 unambiguous and 531 ambiguous) and 95 pairs of loose dihedral restraints (based on α , β , γ , δ and ϵ chemical shifts). f-ACK wraps around Cdc42, forming an extensive interface with an average buried surface area (calculated using NACCESS; S. J. Hubbard and J. M. Thornton) of $\sim 4,200 \text{ \AA}^2$ (Figs 2, 3). The amino terminus of f-ACK interacts with the C terminus of helix α 5 in Cdc42, particularly through hydrophobic contacts between Leu174 in Cdc42 and Leu505 in ACK. Ile510 in f-ACK (the first residue in the CRIB consensus) packs against Ile46 and Leu177 in Cdc42 (conserved hydrophobic residues in Cdc42 and Rac1) at the edge of the three-stranded β -sheet. Residues 515–520 within f-ACK form a strand running antiparallel to residues 41–45 of Cdc42, extending the three-stranded β -sheet. f-ACK then interacts extensively with switch I, fixing its position. The f-ACK chain then forms a hairpin centred around Phe534, and the C terminus, Leu541 and Leu543, packs against Leu67 and Leu70 in switch II of Cdc42. The interacting region of f-ACK includes many residues outside the CRIB region. There are no interactions between f-ACK and the α -helical insert (helix α 3b) which defines the Rho-family proteins¹¹ (Fig. 2).

Much of the structure of Cdc42 is the same in the complex as in

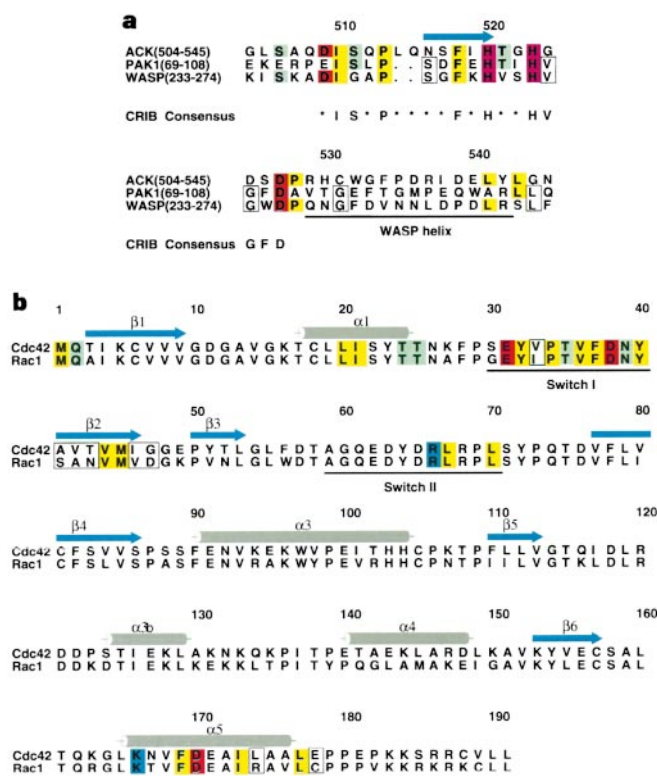


Figure 1 Sequence comparisons, secondary structures and residues involved in complex formation. **a**, Sequence of the Cdc42-binding domain of the ACK tyrosine kinase, showing the homology to other CRIB-domain proteins. Residues that are conserved in WASP and PAK1, but not in ACK, are boxed. The α -helix found in free WASP is indicated by a black line. **b**, Sequence of Cdc42 compared with that of Rac1. Residues that are different where Cdc42 contacts f-ACK are boxed. The residues comprising switch I and switch II are indicated by black lines. In both **a** and **b**, conserved residues involved in the interaction are coloured yellow (hydrophobic), light green (asparagine, glutamine, serine and threonine), red (acidic), magenta (histidine) and dark blue (basic). Blue arrows and grey cylinders indicate the positions of β -strands and α -helices, respectively, in the Cdc42/f-ACK structure. This Figure was produced with Alscript²⁸.

Table 1 Affinities of ACK, PAK and WASP for Cdc42 variants

Cdc42 variant	K_d (nM)		
	f-ACK	PAK1 (75–132)	WASP (201–321)
Q61L	23	23	1.6
Q61L, V42A	540	40	2.5
Q61L, L174A	930	50	29

All measurements were made in a Q61L background to reduce hydrolysis of nucleotide.

Table 2 Structural statistics

	$\langle SA \rangle^*$	$\langle SA \rangle_c^\dagger$
Coordinate precision		
r.m.s.d. of backbone atoms (Å)	1.08 ± 0.11	0.95
r.m.s.d. of all heavy atoms (Å)	1.70 ± 0.11	1.51
(computed over residues 3–120 and 139–179 of Cdc42 and 504–543 of f-ACK)		
r.m.s. deviations		
From the experimental restraints		
NOE distances (Å)	0.027 ± 0.0002	0.026
Dihedral angles (deg)	0.691 ± 0.0680	0.707
From idealized geometry		
Bonds (Å)	$0.002 \pm 7.8 \times 10^{-6}$	0.002
Angles (deg)	0.368 ± 0.0082	0.364
Final energy		
E_{L-J} (kJ mol ⁻¹)	-100 ± 66	-137

* $\langle SA \rangle$ represents the average r.m.s. deviations for the ensemble.

$\dagger \langle SA \rangle_c$ represents values for the structure that is closest to the mean.

‡ The Lennard-Jones potential was not used at any stage in the refinement.

the free protein, but there are important differences. Switch I is fixed in a single conformation and has a lower root-mean-square deviation (r.m.s.d.) in the complex than it does in free Cdc42-GMPPNP (H.R.M. *et al.*, manuscript in preparation). The region of f-ACK that interacts with switch I is also well defined (Fig. 2a). The interaction between Cdc42 and f-ACK therefore leads to the

formation of a high-affinity complex in which unstructured regions of both the effector and the G protein become rigid. In free Cdc42-GMPPNP, helix $\alpha 1$ packs against the three-stranded β -sheet. In the f-ACK complex, this interaction is disrupted by the presence of the β -strand contributed by f-ACK, and the α -helix changes orientation, packing instead against the extra f-ACK strand (Fig. 3a). Switch II is not as well defined as switch I, but it also interacts with f-ACK and has a lower r.m.s.d. in the complex than in free Cdc42 (Fig. 2). In agreement with other solution measurements of Cdc42¹², we do not see a regular α -helix in switch II.

There are similarities between the interactions in the Cdc42/f-ACK complex and those in both Rap1a (a Ras-like G protein) bound to the Ras-binding domain (RBD) from the effector Raf-1⁸, and Ras complexed with the RBD from RalGDS⁹. Both Raf-1 and RalGDS interact by forming an intermolecular β -sheet with the G protein. This interaction may be a common feature in other G-protein/effector complexes. However, the Ras/Rap1a-effector complexes do not involve extensive interactions with residues within the C-terminal α -helix or switch II of the G protein, and the buried surface area is significantly less ($\sim 1,200 \text{ \AA}^2$). Moreover, the Raf-1 and RalGDS RBDs both form compact structures, with a ubiquitin-like fold, and do not wrap around the G protein. Their overall structures are thus very different from that of f-ACK in the Cdc42 complex. These effector interactions are also quite unlike those seen in the Rab and Ran families of small G proteins^{13,14}.

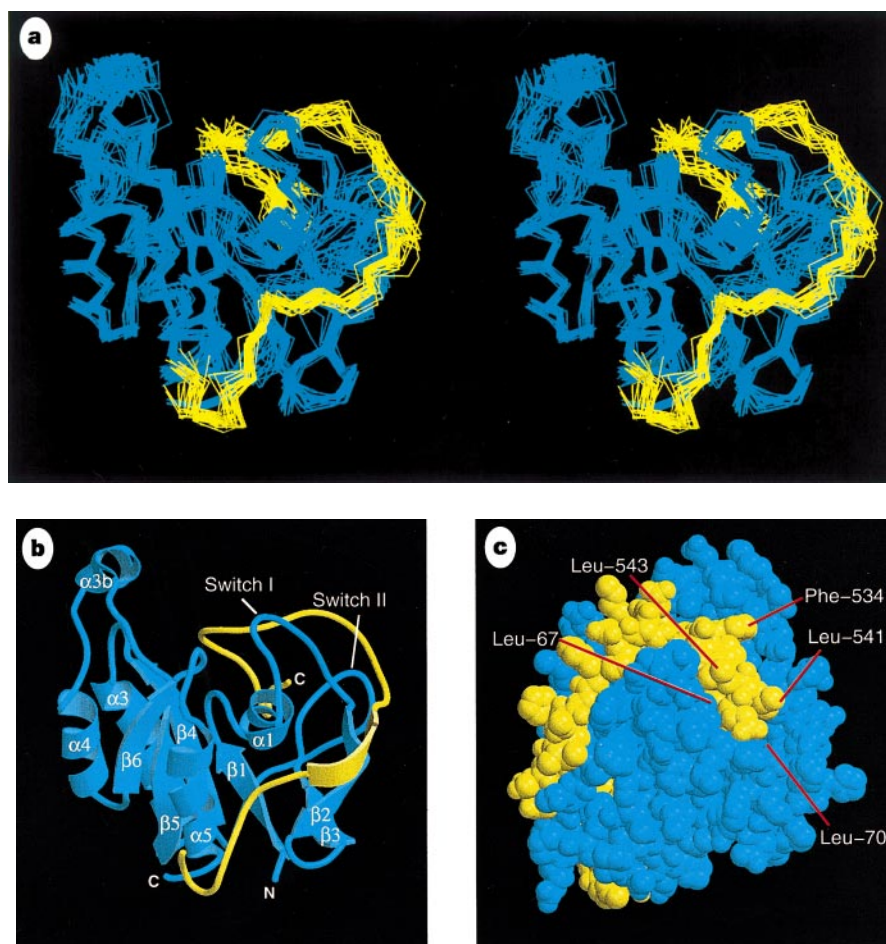


Figure 2 Structure of the Cdc42/f-ACK complex. **a**, Stereoview of the backbone ($C\alpha$ trace) of residues 2–179 of Cdc42 and residues 504–543 of f-ACK from the 20 lowest energy structures (out of 58 that converged from the 100 computed). In the final structures, no distance restraint was violated by more than $0.5 \text{ \AA}$ and no dihedral angle restraint by more than 6.0° . The structures have good covalent geometry and non-bonded contacts (Table 2). **b**, Representation of the structure

closest to the mean in the same orientation as that in **a**. **c**, Space-filled representation of the Cdc42/f-ACK complex, showing the hairpin in ACK and the interactions with switch II. f-ACK wraps around Cdc42 forming an extensive interface, burying a surface area of $\sim 4,200 \text{ \AA}^2$ between the two molecules. The structure is rotated $\sim 120^\circ$ about the z-axis compared to **b**. Figures 2 and 3 were generated using Molscript²⁹ and Raster3D³⁰. Cdc42 is in blue and f-ACK is yellow.

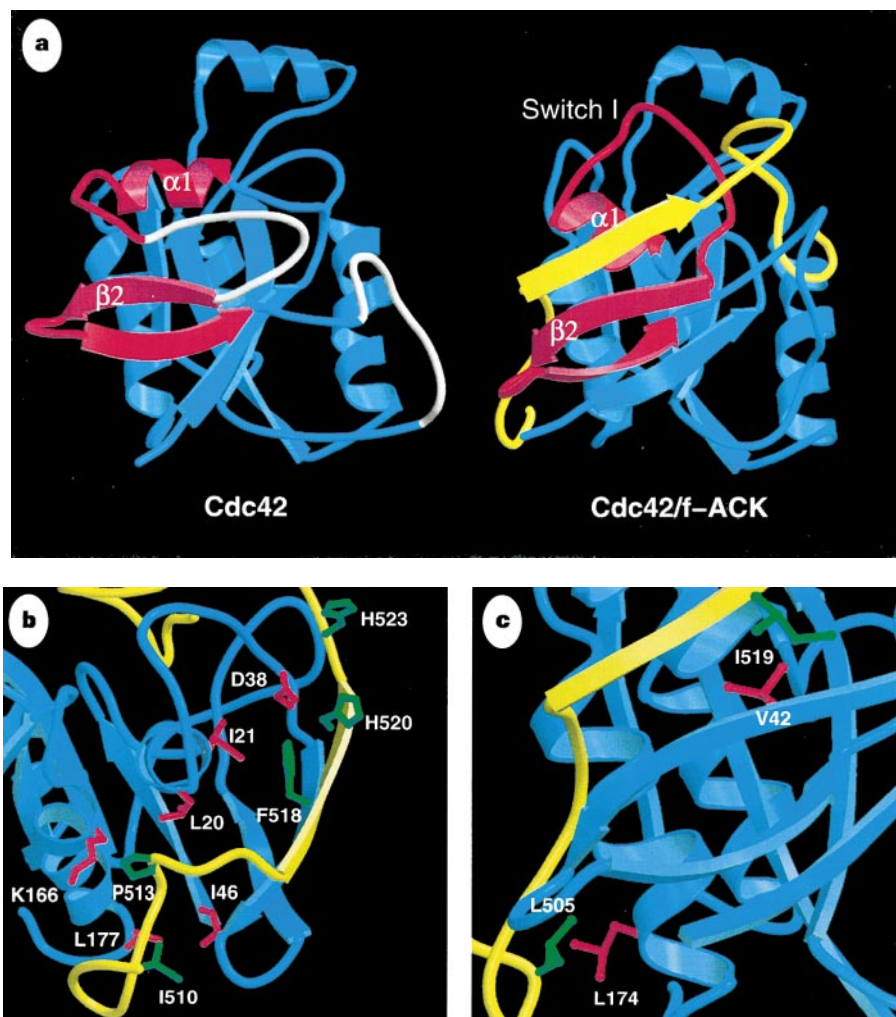


Figure 3 The Cdc42/f-ACK interface. **a**, The positions of switch I and switch II (white in free Cdc42), helix $\alpha 1$ and the β -sheet (red) change upon complex formation. Left, free Cdc42-GMPPNP; right, Cdc42/f-ACK complex. The positions of switches I and II in free Cdc42-GMPPNP are denoted by white lines, because they are flexible and their positions cannot therefore be determined. **b**, **c**, Two

close-up views of the structure shown in Fig. 2b, showing side chains of residues that are likely to provide conserved CRIB-motif/G-protein interactions (**b**), as well as key residues that contribute to the specificity of f-ACK binding to Cdc42 (**c**). Side chains of residues are shown in green (f-ACK) and red (Cdc42).

The structure allows us to understand the role of the conserved residues in the CRIB consensus (Fig. 1a). In addition to those mentioned above, Pro 513 packs against Lys 166 and Leu 20, both of which are conserved in Cdc42 and Rac (Fig. 3b). Phe 518 interacts with both Val 42 and Ile 21 of Cdc42 (Fig. 3b, c). Ile 21 is conserved in Cdc42 and Rac1 and the interaction with Ile 21 in helix $\alpha 1$ probably contributes to the reorientation of that helix. Val 42, however, is not conserved and, because we have found that mutation of Val 42 affects its interactions with ACK but not with PAK or WASP, we infer that the Phe 518/Val 42 interaction is less important than that of Ile 519/Val 42 (Fig. 3b, c). The conserved Asp 38 in Cdc42 and Rac1 interacts mainly with one of the two conserved histidines, His 520 (Fig. 3b). Consistent with this, mutation of Asp 38 to glutamic acid in Cdc42 (ref. 15) or His 208 to aspartic acid in N-WASP¹⁶ greatly affects the affinity of CRIB effector/GTPase interactions.

Some of the regions of Cdc42 that contact f-ACK in the complex have been implicated in binding other proteins. In the Cdc42/rhoGAP complex, the major interactions involve residues 12–15, switch I and switch II of Cdc42. However, there are no interactions with helix $\alpha 5$ or strand $\beta 2$ of Cdc42 (ref. 17). In Rac1 and Cdc42, mutations within switch I reduce its affinity for certain effectors (reviewed in ref. 18), and work with Rac/Rho chimaeras has

implicated residues 143–175 in binding both PAK and the Rac-specific effector p67^{phox} (ref. 19). Other work has implicated the effector loop, the insert region²⁰, residues 103–107 and the C-terminal α -helix, particularly Lys 166 (ref. 21), in p67^{phox} binding. Cdc42 and f-ACK therefore interact in a different manner from Rac1 and p67^{phox}, although the interacting regions overlap, having switch I and the C-terminal α -helix in common.

As mentioned above, f-ACK does not bind to Rac1, despite its 72% sequence identity with Cdc42. Although the effector loop and switch II are highly conserved in the two G proteins, there are differences in other regions that contact f-ACK (Fig. 1b). In particular, Val 42 in $\beta 2$ of Cdc42 makes hydrophobic interactions with Ile 519 in f-ACK (Fig. 3c). In Rac1, this residue is Ala 42, reducing the hydrophobicity of the region. Leu 174 in Cdc42 packs against Leu 505 in f-ACK, forming a second hydrophobic interaction between the two chains (Fig. 3c). In Rac1, residue 174 is an arginine, which would weaken this interaction. The importance of these hydrophobic interactions for f-ACK binding were tested using equilibrium binding assays²² to Cdc42 mutants. Mutation of both Val 42 and Leu 174 in Cdc42 to alanine significantly reduce f-ACK binding (Table 1).

These hydrophobic interactions are also important for the ability of Cdc42 to discriminate between CRIB effectors. In PAK1, which

binds Cdc42 and Rac1, the residue equivalent to Ile519 is a glutamate, indicating that this interaction will be less important for PAK1 binding. Consistent with this, the V42A mutation has little effect on PAK1 binding (Table 1). The residue equivalent to Leu 505 is a lysine in PAK, which will pack less favourably against Leu 174—again, the L174A mutation has very little effect on the affinity for PAK1 (Table 1). Although there is evidence to suggest that PAK does make contact with the C terminus of Cdc42 (ref. 23), our results show that the interaction will be different from that of f-ACK. In contrast, WASP (which is Cdc42-specific) has a hydrophobic group at the residue equivalent to Leu 505 (Fig. 1a) and no longer binds with as high an affinity to the L174A Cdc42 mutant (Table 1). Thus, the interactions of f-ACK and WASP with the C terminus of Cdc42 should be similar. Conversely, the residue equivalent to Ile519 is a lysine, and the V42A mutation of Cdc42 has little effect on its affinity for WASP. This indicates that there will be differences in the Cdc42/f-ACK and Cdc42/WASP interactions. This is also implied by the presence of an α -helix in the free WASP fragment¹⁰. This α -helix is equivalent to residues 529–542 in ACK (Fig. 1a), and there is no evidence for a helix within this region of the Cdc42/f-ACK complex; the C α H resonances up to residue Ile 538 are shifted downfield, indicating that these residues are in an extended conformation. In the remaining seven residues of f-ACK, there is evidence for a single turn of α -helix (residues 540–543), but this is followed in full-length ACK by the sequence Pro-Met-Asp-Pro-Pro, which would not favour continued helix formation. These differences between free WASP and f-ACK indicate that specificity could also be determined by the formation of different structures by the CRIB effectors both in their free forms and in their complexes with Cdc42 and Rac1.

The structure of the Cdc42/WASP complex confirms many of these predictions²⁴. As expected, the binding of the CRIB motif, through the interaction of an extended strand with the β -sheet of Cdc42, is very similar. Outside this region, where the sequences are very divergent, the interactions are also different. The N termini of the two GBDs interact somewhat differently with the C terminus of Cdc42 and, in the Cdc42/ACK complex, the hairpin interacts more closely with Cdc42 than that of WASP. Finally, the C-terminal α -helix in WASP is not present in ACK.

In summary, the structure of a Rho-family protein complexed with an effector domain suggests that novel regions of the G protein are involved in effector interactions. The Rho-family G proteins bind specifically to a large number of effectors, and regions outside the relatively well conserved effector loop contribute to specificity—we suggest that the C-terminal α -helix and the second β -strand of Rac and Cdc42 are specificity-determining regions. In addition, comparison of the interacting regions of both the effectors and the G proteins indicates that the nature of the interfaces will not be the same in all the complexes. □

Methods

Protein expression and purification. We expressed residues 1–184 of human Cdc42 as a His-tagged protein in *Escherichia coli* BL21(DE3). The protein was mutated at position 61 to leucine, to decrease the rate of hydrolysis of GMPPNP. Residues 504–545 of human ACK were expressed as a glutathione S-transferase (GST) fusion protein in *E. coli* BL21. We produced labelled proteins by growing *E. coli* in a medium based on MOPS buffer, containing 5% labelled Celton (Martek), and ¹⁵NH₄Cl and/or ¹³C₆-glucose. Deuterated proteins were produced by growing *E. coli* in medium containing 60% D₂O. We purified fusion proteins on a nickel column (Cdc42) or glutathione-agarose (f-ACK) and cleaved them with factor Xa (Cdc42) or thrombin (ACK), followed by gel filtration on a Superdex-30 column in the case of the ACK fragment. The nucleotide on pure Cdc42 was exchanged to GMPPNP as described²². The Cdc42/f-ACK complex was subsequently purified on a Superdex-75 gel-filtration column in NMR buffer (5 mM Na₂HPO₄, pH 5.5, 25 mM NaCl, 5 mM MgCl₂ and 5 mM DTT) and concentrated with excess GMPPNP (1 mM). **NMR spectroscopy.** Spectra were recorded at 25 °C on Bruker AMX600,

DRX500 and DRX800 spectrometers. Backbone resonances of Cdc42 in the complex were assigned using [50%²H; 100%¹⁵N,¹³C]-labelled Cdc42 and unlabelled f-ACK from HNCA and HN(CO)CA spectra. Side-chain resonances were assigned from a combination of HCCH-TOCSY and ¹⁵N-edited NOESY experiments, recorded using ¹⁵N,¹³C- or ¹⁵N-labelled samples, respectively. Backbone resonances of f-ACK in the complex were assigned using ¹⁵N-edited NOESY and TOCSY spectra of ¹⁵N-labelled f-ACK complexed with unlabelled Cdc42. Side-chain resonances were assigned from an HCCH-TOCSY spectrum of a complex of ¹³C,¹⁵N-labelled f-ACK with unlabelled Cdc42.

Structure calculation. Structures were calculated using X-PLOR²⁵. We calculated initial structures using 2,563 unambiguous NOE restraints, and 91 loose torsion-angle restraints based on chemical shifts. The NOE restraints comprised 560 intra-ACK restraints and 1,900 intra-Cdc42 restraints, derived from ¹⁵N- and ¹³C-separated NOESY experiments recorded on appropriately labelled samples, and 103 intermolecular NOEs derived from the above, and a 2D ¹³C-filtered, ¹³C-edited NOESY spectrum²⁶ recorded on a sample of ¹³C-labelled Cdc42 and unlabelled f-ACK. 531 ambiguous restraints were added to the calculation: of these, 90 were derived from spectra of labelled f-ACK, 418 from labelled Cdc42 spectra and 23 from the ¹³C-filtered, ¹³C-separated experiment. ¹H NMR signals for the nucleotide were assigned using a ¹⁵N X-filtered NOESY spectrum recorded on a ¹⁵N-labelled Cdc42/unlabelled f-ACK sample; this spectrum also provided restraints to Cdc42 (2 unambiguous, 1 ambiguous). Further restraints to the nucleotide (4 unambiguous, 3 ambiguous) were obtained from the ¹³C-separated NOESY experiment recorded using the ¹³C-labelled Cdc42/unlabelled f-ACK sample. The magnesium was modelled in, with octahedral coordination to the side-chain oxygen of Thr 17 and Thr 35, two water molecules and the β - and γ -phosphates of GMPPNP. In addition, we used both intra- and inter-residue NOE restraints from the spectra of free Cdc42–GMPPNP where none of the shifts in the residues involved (¹H, ¹³C or ¹⁵N) changes on complex formation (1,566 unambiguous, 287 ambiguous; 6 unambiguous, 12 ambiguous to the nucleotide). The initial structures were iteratively refined using ARIA methodology²⁷, where the previous set of structures is used to identify restraint violations and to reduce the level of ambiguity in the ambiguous restraint tables by discarding restraints that contribute little to the intensity.

Scintillation proximity assays. These were performed as described²². The concentration of wild-type or mutant Q61L Cdc42–[³H]GTP was varied in the presence of 0.03 μ M GST–ACK, GST–PAK or GST–WASP.

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Correspondence and requests for materials should be addressed to E.D.L. (e-mail: e.d.laue@bioc.cam.ac.uk). The coordinates have been deposited in the Brookhaven PDB (accession number 1cf4).

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A selection of new products for the microbiology lab includes some 45's, not made of black vinyl and not by the Beatles, a manual approach to cryopreservation and ready-to-use media.

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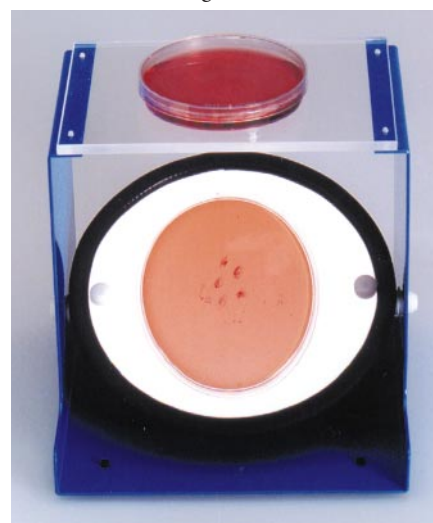
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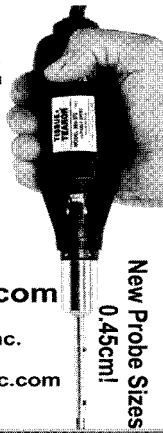
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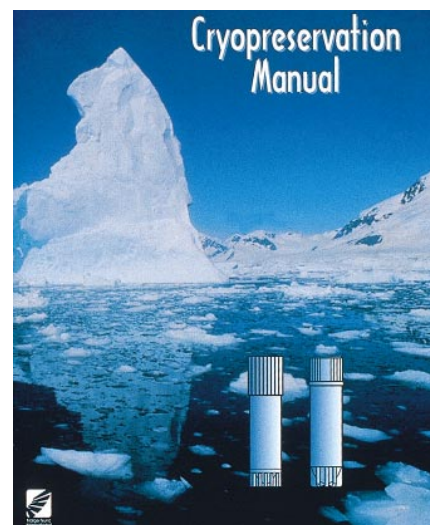
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www.mediators-int.com

A new salmonella test cuts down on detection time

Mediators Diagnostika, based in Vienna, have developed a new diagnostic kit which is claimed to give a dramatic reduction in detection time for the salmonella bacteria in food and other samples. The new hybridization assay can be completed in just 25 hours. Other tests typically require a combined enrichment and detection time of 32 to 48 hours or more. The hybridization process is based on ribosomal RNA (rRNA). rRNA acts as a natural amplifier, with a copy number of around 10,000 per live cell, reducing the number of cells needed for detection and therefore the time it takes to enrich the test sample sufficiently. Furthermore, since RNA degrades rapidly in dead cells, the assay will only detect viable salmonella cells.

Reader Service No. 120

Amplicor Chlamydia

From Roche www.roche.com/diagnostics

A routine test for Chlamydia trachomatis

Responding to the steadily increasing demand for *Chlamydia* screening, Roche Diagnostics has introduced Amplicor

Chlamydia trachomatis to its range for routine testing laboratories. The test is based on a simple standardized PCR and can detect down to as few as 10 copies of *Chlamydia* DNA in a specimen.

Reader Service No. 121

CodaXcel

From Immunetics Inc. www.immunetics.com

Immunoassays in a mere 15 minutes with flow-through technology

The CodaXcel instrument deliver Western blot results for up to 8 samples in only 15 minutes. CodaXcel is fitted with a cassette designed especially to eliminate the need to handle individual strips. Normal Western blots involve adding samples and reagents to strips and removing the fluids with an aspirator. CodaXcel Instrument uses a vacuum to aspirate samples and reagents through the membrane. The instrument is used with CodaXcel test kits, including those for *Borrelia burgdorferi* and HIV.

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